A Morphometrical Analysis of Lymph Node Responses to Tumors of Different Immunogenicity

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ABSTRACT

An immunogenic tumor and a nonimmunogenic tumor were used in C57BL/Ka and CBA/Rij mice, respectively, in a study in which lymph node morphology was quantitatively measured. Lymph nodes were cut in semiseries, and a morphometrical analysis was done on the paracortical and cortical areas as major parts of the active lymph nodes. These parameters could be expressed with confidence in both absolute and relative numbers, since a strong correlation existed between the weight of lymph nodes and the summed surface areas.

To determine whether the regional lymph node plays a major role in this response, we performed similar analyses of two nonregional lymph nodes. It was observed that immunogenicity, defined as the ability to evoke an immune response that significantly influences tumor cell take and tumor cell growth, was not relevant in evoking a morphologically evident response. In absolute numbers, the response evoked was strongest in the regional lymph nodes in both systems. However, the percentages of paracortical and cortical areas in the lymph nodes reacting were not essentially different from one node to another during the growth of both tumors. This observation was confirmed by an analysis of variance. These findings suggest that great caution must be exercised when one is interpreting morphological lymph node changes as evidence of an influence of immunological reactions on tumor cell growth.

INTRODUCTION

Morphological changes in lymph nodes that drain tumors in experimental animals have been studied in considerable detail (1, 2, 5, 9, 12, 18, 29, 31). Experimental models are attractive because they permit sequential and adequately controlled observations. Turk and Heather (35) have pointed out that the immune response as induced by the majority of antigens includes both a humoral and a specific cellular reaction. The deep cortex of the lymph node, which has been called the PCA1 (19), has been shown to be a thymus-dependent area populated mainly by T-lymphocytes (22). This area expands and shows proliferation of large lymphoid cells in the immune response against thymus-dependent antigens (19, 20, 21, 37). Germinal centers have been shown to be thymus-independent regions associated with the production of plasma cells and humoral immune responses (20, 22). This localization of cellular and humoral immunological mechanisms within the lymph node allows a clearer interpretation of the response of the lymph nodes to a tumor.

A standardized system for correlating immunological function with lymph node morphology has been proposed (6). It has also been pointed out that examining the immunological potential of lymph nodes draining a tumor, excised by surgery, should provide further information on prognosis (8). Whether regional lymph nodes that drain a tumor possess properties that make them uniquely different from the rest of the lymphoreticular system is a controversial issue in the literature, especially insofar as preservation or removal of the regional node at an early stage of tumor growth is concerned (3, 7, 10, 11, 14-17, 23-27, 32). Fisher and Fisher (11) stated that a difference in immunogenicity could play an important role. Morphological responses in regional and nonregional lymph nodes that drain experimental tumors of known and different immunogenicity had never been investigated quantitatively. However, an accurate quantification of response can be obtained from histological sections by morphometrical analysis of the PCA’s and CA’s as major parts of the active lymph nodes.

Our purpose was to analyze morphometrically the structural modifications occurring in the regional and nonregional nodes of mice grafted with syngeneic tumors. Attention was focused on 2 main questions: (a) Is there a difference in the morphological aspects of the immune response evoked by an immunogenic and a nonimmunogenic syngeneic tumor? (b) Is there a “staging” in the sense of a serial rather than a simultaneous response in the succeeding nodes of the lymphatic drainage system?

MATERIALS AND METHODS

Tumors and Mice. Mammary adenocarcinoma 2661 originated spontaneously in a CBA/Rij mouse in 1961 and has since been transplanted serially in the flanks of inbred mice of this strain and stored in liquid nitrogen. After inoculation into the footpad, it gives rise to metastatic growth in popliteal, inguinal, paraaortic, and renal lymph nodes as well as in the lungs. For a detailed description of the tumor model and its applications, see the paper of van de Velde et al. (36).

The Lewis lung carcinoma arose spontaneously in the lung of a C57BL mouse in 1951 (33) and was obtained from Professor S. Garattini of the Mario Negri Institute, Milan, Italy, in 1973. It was adapted to C57BL/Ka mice of our institute’s colony. After footpad inoculation, it metastasizes...
always to the lungs but only rarely to lymph nodes.

Tumor cell suspensions were prepared by a modification of the method described by Reinhold (28). Fetal calf serum was not added to the inoculate. For both lymph node morphometry experiments with the mammary carcinoma and the Lewis lung carcinoma, 2 × 10^6 viable tumor cells were inoculated into the footpad in a volume of 0.02 ml with a Hamilton microsyringe. Ten-week-old female CBA/Rij and C57BL/Ka mice were the recipients of the 2 tumor lines described.

Immunogenicity Tests. In previous experiments, the immunogenicity of mammary carcinoma 2661 was determined according to s.c. and i.v. assay techniques (36). These techniques determine either the number of cells needed to obtain a 50% take after s.c. inoculation or the number needed to produce a lung colony after i.v. injection. Neither of these systems showed prior immunization of the recipients to have an effect for this mammary tumor virus-negative tumor (36). The i.v. assay technique used for this assay [Boone et al. (4)] was also used for determining the immunogenicity of the Lewis lung carcinoma in C57BL/Ka mice. Table 1 shows the numbers of lung colonies in immunized and control animals. The difference is highly significant (p < 0.001). A similar effect of immunization on the estimated number of cells necessary to give a tumor take in 50% of inoculation sites (J. H. Mulder, personal communication, 1976) was found on s.c. challenge, indicating that this tumor is immunogenic in its host. It is likely that the immunogenicity of the Lewis lung tumor is due to substrain differences or to the long transplantation history.

Lymph Nodes. Popliteal, inguinal, and paraaortic lymph nodes were removed from 5 animals before, 1 day after, and on every other day until 17 days after injection of the tumor cell suspension. The lymph nodes were fixed in buffered 4% formol, and after removal of fatty tissues their weights were determined on a Mettler type B5 balance. The lymph nodes were then embedded in paraffin and cut into sections 4 µm thick. Every 15th section was retained for the purpose of obtaining semiserial morphology at 200-µm distances. Hematoxylin-eosin staining was used for the morphometrical analysis.

The estimation of the relation between the volume of the PCA, the CA (the lymph follicles), and the volume of the whole lymph nodes by measuring their areas in semiseries was done according to the following formula: volume PCA : volume CA : volume lymph node = summated surface areas PCA : summated surface areas CA : summated surface areas whole lymph nodes.

When different lymph nodes have different sizes during the immune response, the distance between the sections remains the same (200 µm); only the number of serial sections increases. Thus for PCA's: volume PCA lymph node 1 : volume PCA lymph node 2 = summated surface areas 1 : summated surface areas 2. In summary:

\[
\text{Summated surface areas PCA's} = \frac{\text{volume PCA}}{\text{volume lymph node}} \times 100 = \% \text{PCA}
\]

A similar principle was used for the estimation of the CA.

Morphometrical Analysis. Morphometrical determination of the cross-sectional area of entire lymph nodes, lymph node follicles, and PCA's was carried out with a computerized semiautomatic system for direct morphometry on microscopic images. This system consists of a microscope with drawing tubes and a tablet interfaced to a small laboratory computer (PDP12; Digital Equipment Corporation, Maynard, Mass.). The graphic tablet is used for digitizing contour coordinates of figures drawn on the tablet with a capacitive probe. The probe is used for the direct tracing of contours in microscopic images projected via the drawing tubes. A light-emitting diode is mounted in the center of the sensor, which is visible as a small, red spot against a dark background. In this way, contours of objects in the microscopic image can be easily traced manually under visual control. The digitized contours are fed into the computer, which calculates the area and perimeter. The size of the contour on the tablet can be adjusted to a convenient format by using appropriate projection optics in the drawing tubes. The magnification is calibrated with a stage micrometer and used as a correction factor in the computer program. The graphic tablet has a resolution of 0.1 mm. (For a more detailed description of the system and its potentials, see Footnote 3.)

Statistics. All information on the lymph nodes was fed into the computer. For each day of observation and each of the 3 lymph node locations (popliteal, inguinal, and paraaortic), mean and standard deviation of the following parameters were calculated: weight, surface area, surface area of PCA and CA, and percentage of surface of PCA and CA. The course in time for each parameter with its standard deviation was then plotted graphically by the computer. Statistical analysis was used to determine the correlation of weights and surface areas. A variance analysis was done to evaluate differences between the 3 lymph nodes per mouse for each tumor system. The analysis of variance was carried out in the manner described by Scheffé (30). The experiment was regarded as a 3-factor experiment, with the factor "mice" as the random factor and "lymph nodes" and "time" as fixed factors. (Factor mice nested within factor time; factor node crossed with mice and time.) This was done mainly to evaluate the significance or insignificance of interaction between time and lymph nodes.

RESULTS

Correlation between Weights and Summated Surface Areas of Whole Lymph Nodes in the 2 Systems. In both

<table>
<thead>
<tr>
<th></th>
<th>Av. no. of lung colonies a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10) b</td>
<td>44.4 ± 4.77 c</td>
</tr>
<tr>
<td>Immunized (15)</td>
<td>8.7 ± 1.70 c</td>
</tr>
</tbody>
</table>

a p < 0.001 (Student's 2-sample test).
b Numbers in parentheses, number of mice.
c Mean ± SE.

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tumor systems, the weights of all 3 lymph nodes (popliteal, inguinal, and paraaortic) had already increased on the first day after inoculation. This increase continued until Day 17. Chart 1 shows this system, i.e., the 2661 mammary carcinoma system.

The validity and reproducibility of the results of measurements on absolute and relative surface areas in the lymph nodes could be evaluated only from the degree of correlation between weight and summed surface areas of the lymph node cut in semiseries. Chart 2 shows the visual correlation of these 2 parameters during the development of the response to the mammary carcinoma. As shown, there was a good correlation until Days 15 and 17. At that time, lymph node weight increased, whereas total surface area decreased. This was due to ingrowth and destruction of all 3 lymph nodes by metastatic tumor. Tumor area was never included in the morphometrical analysis, and for this reason massive tumor growth in the nodes was expressed by weight increase not associated with surface increase. Since all lymph nodes obviously had to be weighed before histological analysis, there was no longer a good correlation between lymphatic tissue surface area and the weight of the lymph node after its capsule was infiltrated by growing tumor. Consequently, Days 15 and 17 for the mammary carcinoma system were omitted from the results of the morphometrical analysis. Correlation coefficients were calculated for the data obtained up to Day 13. Table 2 shows the results for both the antigenic and the nonantigenic tumors. In both systems, a strong correlation existed between summed surface areas and lymph node weights, suggesting the validity of the method. In particular, it indicated that, even for small volumes, sections at distances of 200 µm warranted a good correlation between the 2 parameters.

Morphometrical Analysis of the PCA’s of the Lymph

Chart 1. Weights of lymph nodes during the growth of mammary carcinoma 2661.

Table 2

<table>
<thead>
<tr>
<th>Lymph node</th>
<th>Lewis lung</th>
<th>Mammary 2661</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popliteal</td>
<td>0.999</td>
<td>0.935</td>
</tr>
<tr>
<td>Inguinal</td>
<td>0.978</td>
<td>0.839</td>
</tr>
<tr>
<td>Paraaortic</td>
<td>0.955</td>
<td>0.995</td>
</tr>
</tbody>
</table>

Nodes Draining the Mammary Adenocarcinoma and the Lewis Lung Carcinoma. Table 3 shows the mean percentages of PCA of the popliteal, inguinal, and paraaortic lymph nodes in the mammary carcinoma 2661 system. There was an initial increase in the percentages of PCA in all 3 lymph nodes, which reached a maximum by Day 3 and then decreased with time. This phenomenon was observed in all 3 subsequent lymph nodes with only minor differences in percentages. However, during the relative decrease, the absolute surface area of the PCA still increased, especially in the regional popliteal lymph node, as shown in the left column for each lymph node. A remarkable finding was that, even when the percentage of the PCA had returned to control values, the absolute size was still increasing. This absolute increase in paraaortal surface area was also found in the antigenic tumor. However, the relative increase and subsequent decrease in the percentages of PCA’s were not as clear as those found in the nonantigenic tumor (Table 4). Both systems (Tables 3 and 4), however, show that the immune response of the PCA’s in absolute numbers

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CA's of the popliteal, inguinal, and paraaortic lymph nodes differed significantly between the 2 tumor systems. Percentages of PCA's of the whole lymph nodes were not significant for lymph node indicated that the course in time and lymph nodes indicated that the course in time was different for the 3 subsequent lymph nodes, i.e., of the 2661 mammary carcinoma was observed. Table 6 shows results for Lewis lung tumor system was observed that there was a staging between the subsequent nodes in the response developing.

Morphometrical Analysis of the CA's of the Lymph Nodes Draining the Mammary Adenocarcinoma and Lewis Lung Carcinoma. Table 5 shows the mean percentages of CA's of the popliteal, inguinal, and paraaortic lymph nodes in the mammary carcinoma system. The maximum response was also found simultaneously in all 3 lymph nodes at the time when the percentage of PCA had decreased to normal or subnormal values (Table 3). This maximum response coincided with the maximum number of germinal centers and follicles per section as shown in Chart 3 for both systems. The latter values were counted visually from every section. In the antigenic tumor, the increase in percentage was strongest in the regional (popliteal) lymph node; they differed significantly between the 2 tumor systems. Percentages of PCA's of the whole lymph nodes were essentially different from one node to another, nor was it observed that there was a staging between the subsequent nodes in the response developing.

Results of the Analysis of Variance. The analysis of variance was performed for each of the following variables: weight of lymph node, surface area of lymph node, surface area of PCA, surface area of CA, PCA as percentage of total surface area, and percentage of CA. For each variable, the following interpretation was applied. A significant interaction of time and lymph nodes indicated that the course in time was different for the 3 subsequent lymph nodes, i.e., popliteal, inguinal, and paraaortic lymph nodes. A significant value for lymph node indicated differences in mean over time for the 3 subsequent lymph nodes. A significant value for time indicated that the mean of the 3 lymph nodes changed with time.

Table 7 shows the results of this analysis. As shown for the mammary carcinoma, there was no significant interaction of time and lymph nodes in percentages of PCA and CA, whereas all time and lymph node values were significant. This indicated that both the absolute and relative areas of PCA and CA had their own identity for node, point in time, and tumor system. However, the course in time of the changes in percentages of PCA and of CA was similar in all 3 lymph nodes. It appears, in spite of the slight interaction in percentage of CA for the Lewis lung system (5%), that the same was true in this essentially different system. This suggests a generalized response of thymus-dependent and -independent immune systems as a reaction to the growing tumors.

DISCUSSION
Two tumor systems of spontaneous origin were used in the present study to evaluate differences in the response in the lymph nodes. Following s.c. and i.v. immunity challenge tests, the mammary carcinoma was found to be nonimmunogenic and the Lewis lung carcinoma was found to be immunogenic. There was no effect of prior immunization for the mammary carcinoma, whereas for the Lewis Lung carcinoma there was a significant effect on the estimated...
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Table 5
Mean of summated surface areas of all cross-sections and calculated mean percentages of CA's during the growth of the 2661 mammary carcinoma

<table>
<thead>
<tr>
<th>Size (sq mm)</th>
<th>%</th>
<th>Size (sq mm)</th>
<th>%</th>
<th>Size (sq mm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.17 ± 0.08²</td>
<td>18.0 ± 10.0</td>
<td>Day 1</td>
<td>0.26 ± 0.17</td>
<td>17.6 ± 11.7</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.52 ± 0.27</td>
<td>16.0 ± 9.6</td>
<td>Day 3</td>
<td>1.40 ± 0.67</td>
<td>30.0 ± 12.5</td>
</tr>
<tr>
<td>Day 5</td>
<td>1.10 ± 0.63</td>
<td>23.8 ± 13.1</td>
<td>Day 5</td>
<td>1.86 ± 0.41</td>
<td>37.4 ± 4.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>2.00 ± 0.67</td>
<td>31.4 ± 11.8</td>
<td>Day 7</td>
<td>2.38 ± 0.79</td>
<td>45.8 ± 5.0</td>
</tr>
<tr>
<td>Day 9</td>
<td>3.96 ± 1.48</td>
<td>31.0 ± 9.5</td>
<td>Day 9</td>
<td>2.32 ± 1.23</td>
<td>43.2 ± 11.8</td>
</tr>
<tr>
<td>Day 11</td>
<td>7.58 ± 1.85</td>
<td>48.0 ± 15.7</td>
<td>Day 11</td>
<td>2.88 ± 0.89</td>
<td>44.2 ± 7.0</td>
</tr>
<tr>
<td>Day 13</td>
<td>5.84 ± 1.07</td>
<td>14.0 ± 5.5</td>
<td>Day 13</td>
<td>2.91 ± 1.22</td>
<td>50.2 ± 10.3</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

**Chart 3. Mean number of germinal centers per section of the lymph nodes. A, mammary carcinoma 2661; B, Lewis lung tumor.**

The number of cells necessary to give a tumor take in 50% of inoculation sites and the number of lung colonies. When inoculated into the footpad, both tumors evoked responses in regional and nonregional lymph nodes, so that the weights of the lymph nodes were already increased on the first day after inoculation, and this continued until the end of the observation period. The weights of the lymph nodes correlated visually and statistically with the calculated summated surface of the lymph node sections; this allowed us to make calculations of the percentages of PCA and CA within the lymph node. The increased accuracy of this method of morphometrical quantification over visual estimation of the sizes of different parts within the lymph nodes is evident, thus opening many opportunities for the application of statistics. The reaction patterns of the percentages of PCA in the nonimmunogenic mammary carcinoma system showed an early increase and subsequent decrease in time. This percentage of decrease in PCA coincided with an increase in the percentage of CA and in the number of germinal centers. A similar pattern was observed for the immunogenic Lewis lung carcinoma. These similar patterns indicate that immunogenicity, as measured by the effect of transplantation resistance, of the tumors is not an essential factor in producing different morphological reactions in
which sizes of different parts of lymph nodes were estimated, i.e., the percentages of PCA and CA of the 3 lymph nodes.

The course in time is similar. This visual correlation of the morphological findings, these results are in agreement with the findings of Flannery et al. (13), who found that only regional lymph nodes have a reaction pattern similar to that described earlier.

In view of the controversy in the literature, we feel that a quantitative approach is essential in analyzing the morphological response. Immunogenicity, defined as the ability to evoke an immune response that induces significant effects on tumor cell take and tumor cell growth, was not relevant in evoking a morphological response. The absolute value of total node area and weight reached a peak response which the 2661 mammary tumor developed at different rates has suggested that the growth rate may also be a factor that determines surface area response (unpublished observations). We conclude from these findings that typical morphological changes in the lymph nodes cannot with certainty be interpreted as evidence of an influence of immunological reactions on tumor cell growth. It is not even quite clear what part of the reactivity of the lymph nodes and, despite the quantification of the lymphoreticular system and whether it should be preserved or removed with a primary tumor. Experimental data on the effect of lymph node removal are conflicting. Some find a detrimental effect of early removal (7, 10, 11, 15, 23, 25, 26), whereas others did not find such an effect (3, 14, 16, 17, 24, 27, 32). Fisher (11) suggested that the regional lymph node may play a more significant role in influencing tumor growth in tumors of "low" immunogenicity. Since both an immunogenic tumor and a nonimmunogenic tumor were used in this study, this could not be confirmed on a morphological basis. The special properties found for the regional lymph node were quantitatively but not qualitatively different from "distant" lymph nodes evaluated. Treves et al. (34) observed an increase in spleen weight as early as 2 days after implantation of the Lewis lung carcinoma into the footpad. This suggests that the response by that time is already beyond the lymph nodes.

In this regard, our findings are in agreement with the findings of Simar (31) and Edwards (9), who examined the morphological alterations of contralateral lymph nodes in experimental studies. In contrast, however, are the studies of Alexander et al. (2), Carter and Gershon (5), and Fisher et al. (12), who found that only regional lymph nodes have a reaction pattern similar to that described earlier.

### Table 6

**Mean of summed surface areas of all cross-sections and calculated mean percentages of CA’s during the growth of the Lewis lung tumor**

<table>
<thead>
<tr>
<th></th>
<th>Popliteal</th>
<th>Inguinal</th>
<th>Paraortic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size (sq mm)</strong></td>
<td>%</td>
<td>Size (sq mm)</td>
<td>%</td>
</tr>
<tr>
<td>Controls</td>
<td>0.11 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.8 ± 9.0</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.20 ± 0.09</td>
<td>18.2 ± 5.6</td>
<td>0.70 ± 0.10</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.73 ± 0.18</td>
<td>39.6 ± 6.4</td>
<td>1.51 ± 0.33</td>
</tr>
<tr>
<td>Day 5</td>
<td>1.30 ± 0.13</td>
<td>45.8 ± 14.1</td>
<td>1.26 ± 0.63</td>
</tr>
<tr>
<td>Day 7</td>
<td>2.01 ± 0.44</td>
<td>41.2 ± 5.0</td>
<td>1.85 ± 0.74</td>
</tr>
<tr>
<td>Day 9</td>
<td>2.14 ± 1.30</td>
<td>29.2 ± 16.8</td>
<td>2.46 ± 1.01</td>
</tr>
<tr>
<td>Day 11</td>
<td>4.51 ± 1.16</td>
<td>28.0 ± 6.5</td>
<td>2.57 ± 1.30</td>
</tr>
<tr>
<td>Day 13</td>
<td>6.78 ± 1.82</td>
<td>52.0 ± 25.7</td>
<td>2.78 ± 2.16</td>
</tr>
<tr>
<td>Day 15</td>
<td>5.84 ± 2.95</td>
<td>30.4 ± 11.9</td>
<td>4.31 ± 2.75</td>
</tr>
<tr>
<td>Day 17</td>
<td>13.06 ± 5.02</td>
<td>35.0 ± 12.9</td>
<td>8.06 ± 4.06</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D.

### Table 7

**Results of the analysis of variance**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mammary carcinoma</th>
<th>Lewis lung carcinoma</th>
<th>Time&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Lymph node</th>
<th>Time&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node wt</td>
<td>45.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area whole lymph node</td>
<td>57.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area PCA</td>
<td>28.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area CA</td>
<td>20.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% PCA</td>
<td>9.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>% CA</td>
<td>10.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewis lung carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node wt</td>
<td>70.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area whole lymph node</td>
<td>55.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface area PCA</td>
<td>34.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Surface area CA</td>
<td>18.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% PCA</td>
<td>3.57&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% CA</td>
<td>9.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.22&lt;sup&gt;d&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> Up to Day 13.

<sup>b</sup> Significant at 1% level.

<sup>c</sup> Not significant at 5% level.

<sup>d</sup> Significant at 5% level.
nodes must be attributed to such nonspecific factors as cell necrosis and nonspecific inflammation in the tumor. A quantitative approach such as a morphometrical analysis with the possibility of applying statistics may be an improvement in evaluating responses in lymph node studies.

REFERENCES


MARCH 1978
A Morphometrical Analysis of Lymph Node Responses to Tumors of Different Immunogenicity


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