Lymph Node Reaction to Cancer

(Immunohistochemical and Ultrastructural Study)

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A total of 153 regional lymph nodes obtained from 50 patients, operated for gastric, lung, breast, colonic and cervical cancers, were studied. Immunohistochemical methods were used to detect different markers and enzymes (CD1, CD2, CD3, CD4, CD8, CD20, CD30, CD35, CD45, \( \lambda \) light Ig chain, lysozyme (muramidase), \( \alpha \)-1-antichymotrypsin, protein S100 and FVIIIIR). Results indicate that failure of local immunity is explained by the following: 1. decrease in the total number of T-cells (suppressors as well as helpers); 2. high number of B-cells, plasmablasts and antibody-forming plasmacytes, know to be able to block the cytotoxic T cells; 3. decrease in the number of incoming free phagocytes of monocyctic origin and reduction in the phagocytic activity of fixed macrophages (sinus histiocytes); 4. high functional activity of dendritic reticulum cells; 5. non-handled stimulation of T cell response by the paracortical interdigitating reticulum cells; 6. reduction in area of postcapillary venules and impairment of lymphocyte recirculation through them. (Pathology Oncology Research Vol 3, No 2, 121–125, 1997)

Key words: lymph node, cancer, immunohistochemistry

Introduction

The state of lymph nodes draining a tumor can profoundly influence tumor progression and largely determines the survival of cancer patients.4,5 Despite the large number of studies available so far,2A,1,12A,25 there is no agreement yet on the role of the barrier function of the lymph nodes. Some authors regard lymphadenectomy as a factor precluding cancer dissemination, others claim that it results in a decrease in antitumoral resistance. They suggest that local administration of some immunostimulating agents could enhance the cytotoxic activity of the lymph nodes.

The aim of this immunohistochemical and ultrastructural study was to examine normal lymph nodes and those involved in cancer progression, as well as to determine factors contributing to the deficiency of the local immune response and to the tumor progression.

Material and Methods

A total of 153 regional lymph nodes obtained from 50 patients operated because of gastric (41), lung (29), breast (27), colonic (25) and cervical (31) cancer were studied. Fifty lymph nodes from identical regions in 10 healthy persons, who died as a result of accidents, were used as “controls” (autopsy was performed no later than 12 hours after death).

Following an adequate histological procedure9 the immunohistochemical analysis was made with monoclonal antibodies from BioGenex, Dako and Sigma. Using peroxidase-antiperoxidase (PAP), biotin-streptavidin (BSA) or immunofluorescent methods, the total number of CD45+ leukocytes, CD10+ lymphoblasts, CD2+ and CD3+ T-lymphocytes, CD4+ T-helpers/inducers, CD8+ T-killers/suppressors, CD30+ activated lymphocytes, CD20+ B-lymphocytes, anti-\( \lambda \) light chain positive plasma cells, lysozyme (muramidase) positive free macrophages, \( \alpha \)-1-antichymotrypsin (AAST)+ fixed macrophages (sinus histiocytes), CD1+ and CD35+ follicular dendritic reticulum cells, CD1+ and S100+ interdigitating paracortical reticulum cells, FVIIIIR+ endothel-
Table 1. Morphometric immunophenotypes of lymph node cells in cancer patient (%; M±m)

<table>
<thead>
<tr>
<th></th>
<th>I Group</th>
<th>II Group</th>
<th>III Group</th>
<th>IV Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45+ leukocytes</td>
<td>86.4±2.76</td>
<td>89.54±1.95</td>
<td>88.41±2.35</td>
<td>84.82±2.54</td>
</tr>
<tr>
<td>CD10+ lymphoblasts</td>
<td>1.74±0.23</td>
<td>4.96±0.34*</td>
<td>2.58±0.14*</td>
<td>0.34±0.1 abc</td>
</tr>
<tr>
<td>follicles</td>
<td>5.21±0.31</td>
<td>8.65±0.74*</td>
<td>9.21±0.79</td>
<td>7.94±0.28*</td>
</tr>
<tr>
<td>CD30+ activated lymphocytes</td>
<td>4.52±1.01</td>
<td>10.53±0.86</td>
<td>7.42±0.85*</td>
<td>3.62±0.3 abc</td>
</tr>
<tr>
<td>CD2+ and CD3+ T cells</td>
<td>38.91±1.89</td>
<td>45.75±2.91</td>
<td>37.45±1.79</td>
<td>29.6±1.5 ab</td>
</tr>
<tr>
<td>CD4+ helper/inducer T cells</td>
<td>25.42±0.68</td>
<td>33.96±1.74</td>
<td>29.71±0.94</td>
<td>21.6±0.8 abc</td>
</tr>
<tr>
<td>CD8+ killer-suppressor T cells</td>
<td>11.15±0.65</td>
<td>15.92±0.56</td>
<td>10.45±0.51</td>
<td>8.61±0.1 abc</td>
</tr>
<tr>
<td>CD20+ B cells</td>
<td>21.46±0.94</td>
<td>24.16±0.90</td>
<td>25.12±1.02</td>
<td>20.9±0.65 abc</td>
</tr>
<tr>
<td>CD1+ and S100+ IRC</td>
<td>2.48±1.30</td>
<td>11.62±0.41</td>
<td>12.51±0.56</td>
<td>9.86±0.5 abc</td>
</tr>
<tr>
<td>l light lg chain + plasma cells</td>
<td>1.01±1.06</td>
<td>4.23±0.10*</td>
<td>3.96±0.11</td>
<td>0.87±0.04 abc</td>
</tr>
<tr>
<td>lysozyme + free macrophages</td>
<td>38.61±1.04</td>
<td>41.01±1.10</td>
<td>40.77±2.98</td>
<td>35.4±1.1 abc</td>
</tr>
<tr>
<td>AAST + fixed macrophages</td>
<td>23.17±1.35*</td>
<td>25.24±1.47</td>
<td>29.2±0.3 abc</td>
<td></td>
</tr>
<tr>
<td>CD1+ and CD35 + DRC</td>
<td>18.71±0.97</td>
<td>19.42±0.85</td>
<td>18.56±0.17</td>
<td>17.62±1.04</td>
</tr>
</tbody>
</table>

AAST: α1-antichymotrypsin; IRC: interdigitating reticulum cells; DRC: dendritic reticulum cells
p<0.05 – a: between I and II, III, IV groups; b: between II and III, IV groups; c: between III and IV groups

Results

The progression of cancer was accompanied by a certain increase in the total number of CD45+ cells (Table 1). In the hyperplastic paracortical zone (Table 2), as expected, the number of CD2+ and CD3+ T cells – both CD4+ helpers/inducers and CD8+ killers/suppressors – was elevated (Fig. 1). The number of CD10+ lymphoblasts and CD30+ activated lymphocytes, as well as the number of CD1+ and S100+ interdigitating reticulum cells also increased. These cells are responsible for antigenic stimulation of T-lymphocytes. Lysozyme (muramidase) positive free macrophages were present in large number. Proliferation of VVIIIR positive postcapillary venules (Fig. 2), through which lymphocytes recirculate, was also pronounced. Ultrastructural study showed that lymphoblasts, which are identified immunohistochemically as CD10+ cells, appeared to be T-lymphocyte in the paracortical zone, in which ribosomes were not related to endoplasmic reticulum. The growing number of CD30+ and CD8+ lymphocytes is also in agreement with the results of electron microscopic studies. All of these can be considered as evidences for an enhanced cellular immune response in patients with stage I cancer.

Humoral immune reaction took place at the same stage of tumor growth; lymphoid follicles became hyperplastic with increased amount of CD1+ and CD35+ dendritic reticulum cells. The latters are known to be responsible for antigenic stimulation of B cells and have a common,

Table 2. Morphometric volume of some lymph node structures in cancer patients (%; M±m)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>paracortex</td>
<td>6.21±0.91</td>
<td>18.77±1.02*</td>
<td>17.24±0.38*</td>
<td>7.43±0.36 abc</td>
</tr>
<tr>
<td>follicles</td>
<td>1.24±0.02</td>
<td>11.45±0.86*</td>
<td>12.48±0.93*</td>
<td>10.61±0.16*</td>
</tr>
<tr>
<td>sinuses</td>
<td>15.25±0.83</td>
<td>26.76±1.38*</td>
<td>23.61±0.95*</td>
<td>16.14±0.98 abc</td>
</tr>
<tr>
<td>postcapillary venules</td>
<td>1.86±0.12</td>
<td>3.01±0.11*</td>
<td>2.70±0.24 abc</td>
<td>1.50±0.11 abc</td>
</tr>
</tbody>
</table>

p<0.05 – a,b,c – see in Table 1.
CD20, differentiating antigen. Blastic transformation and antibody-production were observed in the mantle zone of follicles. The percentage of antibody-forming plasma cells was elevated. Ultrastructural study also revealed plasmablast transformation (here, unlike in T lymphoblasts, ribosomes are connected with endoplasmic reticulum) (Fig. 3). In dendritic reticulum cells, the number of organelles increased in the cytoplasm, multiple contacts were observed between the cytoplasmic processes and lymphocytes. During tumor progression the macrophage-phagocytic system was also activated showing sinus histiocytosis. The enlarged sinuses were rich in AAST+ reticulum cells and lysozyme (muramidase) positive free macrophages.

Essential changes took place in the lymph nodes not involved in the metastatic process, when disease reached stage II. Although, the area of the paracortical zone as well as the total number CD45+ cells remained constant, the number of CD2+ and CD3+ lymphocytes decreased, which was largely due to the decrease of CD8+ cells. Again, the number of CD1+ and S-100+ interdigitating reticulum cells was constant, the number of CD10+ lymphoblasts, CD30+ activated lymphocytes decreased, similarly to the area of FVIIIIR positive postcapillary venules. These results were confirmed at the ultrastructural level: T lymphoblasts and transformed immune cells as well as intercellular contacts became more sparse. The postcapillary venules often looked as desolated, with no signs of lymphocyte migration. At the same time the intensity of humoral immune reactions was preserved in stage II.

In stage II HE staining showed markedly enlarged sinuses filled with different cells, however the prognostic significance of this “sinus histiocytosis” is conflicting. A better estimation is offered by the recognition of changes in cell types. In fact, despite the unchanged total cellularity, there was a decrease in lysozyme positive free macrophages, redistribution of lymphocyte population with an increase of CD20+ B-cells at the expense of CD2+ and CD3+ T cells. Concerning reticulum cells, total number of AACT positive forms remained constant, but their phagocytic activity went down. The latter was reflected at the ultrastructural level. The nucleus of such cells became more electron dense, without invaginations, the cytoplasmic area diminished as well as the number of cytoplasmic processes, ribosomes and large mitochondria. On the whole, the cells took an elongated form and could be defined as fibroblastic reticulum cell. Besides, collagen synthesizing true fibroblasts also appear in the sinuses.

In metastatic lymph nodes the area of the paracortical zone decreased together with the total number of CD2+ and CD3+ T cells, including both CD4+ and CD8+ subpopulations. Our data do not support the hypothesis that the number of suppressor cells is increasing during

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**Figure 1.** Immunostaining of CD3+ T-cells in hyperplastic paracortical area. DAB, no counterstain. x70

**Figure 2.** Immunostaining of FVIIIIR positive postcapillary venules in paracortical area. DAB with hematoxylin counterstain. x200.

**Figure 3.** Detail of a plasmablast with rough endoplasmic reticulum. EM, x12000.
Conclusions

Several factors indicate the deficiency of the local immune response in the tumor-draining lymph nodes: a. decrease in total number of T cells, T lymphoblasts, even at the early stage of tumor growth; b. high number of B-cells, plasmoblasts and antibody-producing plasmocytes (humoral antibodies are able to block cytotoxic effects of T cells at all stages of tumor progression); c. insufficiency of suppressor T cells to inhibit various immunoglobulins; d. decrease in the number of helper T cells makes difficult the activation of cytotoxic lymphocytes and macrophages; but has no effect on the activation of B-cells; e. decrease in the number of mobile macrophages and in the phagocytic activity of fixed macrophages (sinus histiocytes); f. high functional activity of dendritic reticulum cells which process the antigens for B cells; g. non-regulated stimulation of T cells’ immunity by paracortical interdigitating reticulum cells; h. reduction of the area of postcapillary venules and impairment of lymphocyte recirculation.

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References