

ARTICLE

Lymph Node Reaction to Cancer

(Immunohistochemical and Ultrastructural Study)

Dmitry E TSYPLAKOV¹, Semion V PETROV,² Roman N KULAGIN¹

¹Department of Pathology, Medical University; ²Clinical Oncological Centre, Kazan, Russia

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, λ light Ig chain, lysozyme (muramidase), α -1-antichymotrypsin, protein S100 and FVIIIIR). Results indicate that failure of local immunity is explained by the followings: 1. decrease in the total number of T-cells (suppressors as well as helpers); 2. high number of B-cells, plasmoblasts and antibody-forming plas-

mocytes, know to be able to block the cytotoxic T cells; 3. decrease in the number of incoming free phagocytes of monocytic 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,^{2,6,13,14,15} 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 procedure¹⁹ 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- λ light chain positive plasma cells, lysozyme (muramidase) positive free macrophages, α_1 -antichymotrypsin (AAST)+ fixed macrophages (sinus histiocytes), CD1+ and CD35+ follicular dendritic reticulum cells, CD1+ and S100+ interdigitating paracortical reticulum cells, FVIIIIR+ endothe-

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Correspondence: Dmitry E TSYPLAKOV, M.D., Department of Pathology, Medical University¹; Clinical Oncological Centre², Butlerov str 49, Kazan 420012, Russia; Fax: 007-8432-360393

Table 1. Morphometric immunophenotypes of lymph node cells in cancer patient (% , M±m)

	I Group	II Group	III Group	IV Group
CD45+ leukocytes	86.41±2.76	89.54±1.95	88.41±2.35	84.82±2.54
CD10+lymphoblasts	1.74±0.23	4.96±0.34 ^a	2.58±0.14 ^{ab}	0.34±0.1 ^{abc}
follicles	5.21±0.31	8.65±0.74 ^a	9.21±0.79 ^a	7.94±0.28 ^a
CD30+ activated lymphocytes	4.52±1.01	10.53±0.86	7.42±0.85 ^{ab}	3.62±0.3 ^{bc}
CD2+ and CD3+ T cells	38.91±1.89	45.75±2.91	37.45±1.79 ^b	29.6±1.5 ^{ab}
CD4+ helper/inducer T cells	25.42±0.68	33.96±1.74	29.71±0.94	21.6±0.8 ^{abc}
CD8+ killer/suppressor T cells	11.15±0.65	15.92±0.56	10.45±0.51 ^b	8.61±0.1 ^{abc}
CD20+ B cells	21.46±0.94	24.16±0.90	25.12±1.02 ^a	20.9±0.6 ^{bc}
λ light Ig chain + plasma cells	2.48±1.30	11.62±0.41	12.51±0.56	9.36±0.5 ^{abc}
lysozyme + free macrophages	1.01±0.06	4.23±0.10 ^a	3.96±0.11 ^a	0.87±0.04 ^b
AAS + fixed macrophages	38.61±1.04	41.01±1.10	40.77±2.98	35.4±1.1 ^{abc}
CD1+ and S100 + IRC	14.26±0.21	23.17±1.35 ^a	25.24±1.47 ^a	29.2±0.3 ^{abc}
CD1+ and CD35 + DRC	18.71±0.97	19.42±0.85	18.56±0.17	17.62±1.04

AAS: 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

lial cells of postcapillary venules, were detected. Specimens for ultrastructural study were treated according to the standard methods and studied under JEM-100C electronmicroscope. Additionally, cryostate and paraffin sections were stained with hematoxyline and eosin, Brashe's pyronine and azure-II-eosin. The quantitative evaluation of cellular and structural elements was made using a morphometric net¹ and with statistical analysis of the data. To analyse tumor progression 4 groups were selected: 1. controls (50 lymph nodes); 2. lymph nodes with no metastasis in the stage I cancer (68); 3. lymph nodes with no metastasis in stage II cancer (33); 4. lymph nodes with metastasis in stages II and III (52). Staging was made according to TNM classification.

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 ele-

vated (Fig.1). The number of CD10+ lymphoblasts and CD30+ activated lymphocytes, as well as the number of CD1+ and S-100+ interdigitating reticulum cells also increased. These cells are responsible for antigenic stimulation of T-lymphocytes¹⁸. Lysozyme (muramidase) positive free macrophages were present in large number. Proliferation of FVIIR 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-blast 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.³ 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 latter are known to be responsible for antigenic stimulation of B cells¹⁸ and have a common,

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

	I Group	II Group	III Group	IV Group
paracortex	6.21±0.91	18.77±1.02 ^a	17.24±0.38 ^a	7.43±0.36 ^{bc}
follicles with reactive centers	1.24±0.02	11.45±0.86 ^a	12.48±0.93 ^a	10.61±0.16 ^a
sinuses	15.25±0.83	26.76±1.38 ^a	23.61±0.95 ^a	16.14±0.98 ^{bc}
postcapillary venules	1.86±0.12	3.01±0.11 ^a	2.70±0.24 ^{ab}	1.50± 0.11 ^{abc}

p<0.05 – a,b,c – see in Table 1.

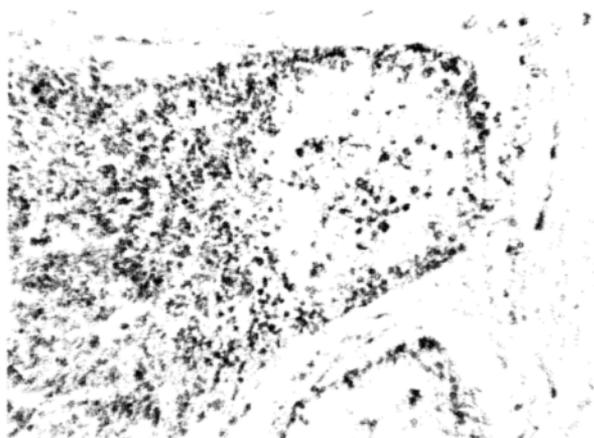


Figure 1. Immunostaining of CD3+ T-cells in hyperplastic paracortical area. DAB, no counterstain, x70

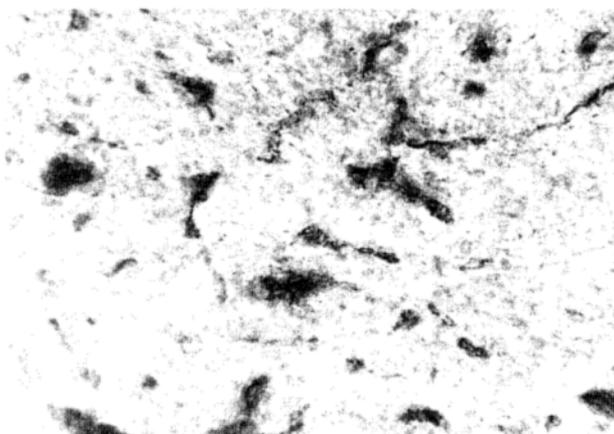


Figure 2. Immunostaining of FVIIR positive postcapillary venules in paracortical area. DAB with hematoxylin counterstain, x200.

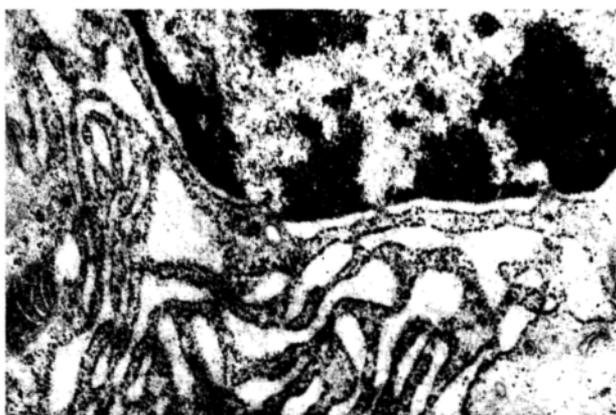


Figure 3. Detail of a plasmoblast with rough endoplasmic reticulum. EM, x12000.

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 plasmoblast 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.⁸ 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 FVIIR+ 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

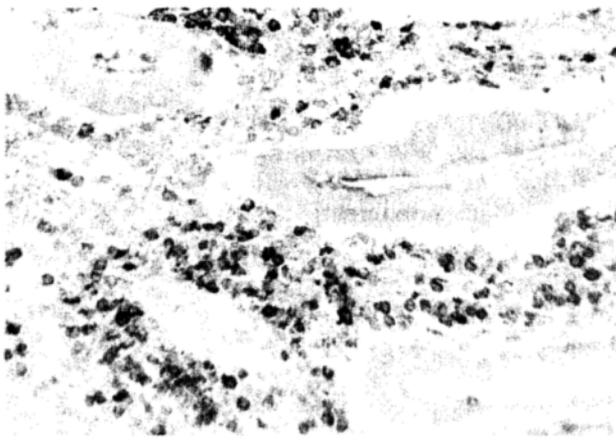


Figure 4. Lymph node metastasis of an adenocarcinoma. Immunostaining of λ light Ig chain expressing plasma cells around the tumor cells. DAB with hematoxylin counterstain, x200.

metastatic process.¹¹ Accordingly, the number of CD30+ activated lymphocytes and CD10+ lymphoblasts decreased. Lysozyme positive free macrophages were rather rare. With tumor progression the number of CD1+ and S100+ interdigitating reticulum cells was elevated, as it was also observed by others.^{9,10} This phenomenon apparently indicates the attempt of the immune system to realize the antigen stimulation of the inactivated T-cell immunity, without success. Furthermore, in the paracortical zone the electron microscopy revealed together with non-transformed T cells a large number of degenerating cells with pyknotic nucleus and destroyed structures in the vacuolized cytoplasm.

When metastases develop, sinuses are subject to drastic structural and functional changes, which is evident even with routine staining. As a rule, the area of sinuses is significantly decreasing. If they remain enlarged, depletion of cellular elements is observed in the presence of lymphostasis. There is an almost complete disappearance of free macrophages and decrease in phagocytic activity of fixed histiocytes, and a reduction in the number of T cells and activated lymphocytes. Interestingly, most of the remaining cells seem to be B lymphocytes.^{16,17} Decrease of neutrophils should also be mentioned. Besides, at this stage, sclerosis of sinuses was found, with a great number of active fibroblasts.

Thus, tumor progression was accompanied by the inactivation of the macrophage-monocyte system and cellular immunity, but it seemed that humoral immune reactions were maintained. Follicles with large reactive centers were constantly present, and there was no decrease in the total number of B cells and plasma cells (Fig.4). Such B-cell (plasmocytic) reaction remained unchanged almost till complete substitution of lymph node with tumor cells.

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 antibodies⁷ 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;¹² 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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