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HISTOMORPHOLOGIC REACTION PATTERNS IN CERVICAL LYMPH NODES OF DIFFERENT NECK SITES*

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ABSTRACT

Immunologically based studies of host-tumor interactions have gained popularity in head and neck oncology and histopathological determination of lymph node reactivity has been shown to correlate with survival. However, little is known about lymph node reaction patterns in the "normal" neck. In a prospective histomorphologic study 1024 cervical lymph nodes (CLN) were dissected at autopsy from 25 selected cadavers free of head and neck disease. To investigate regional differences of immune reactivity, these lymph nodes were grouped according to neck levels. 242 CLN groups of 50 neck sites were microscopically classified into one of four distinct histomorphologic immune reaction patterns (RP). 14.5% of CLN groups showed lymphocyte predominance (RP 1), 12% showed germinal center predominance (RP 2), 63.2% were unstimulated (RP 3) and 10.3% displayed regressive changes (RP 4). Distribution of RP was highly significant according to neck level ($p<0.001$): RP 1 and RP 2 were common in the submandibular (Level I) and the upper parajugular groups (Level II) whereas RP 4 was more typical in the inferior parajugular groups (Level IV) and posterior triangle (Level V). RP 2 and RP 4 showed significant correlation to age and general condition. These findings suggest that tumor independent lymph node reactivity related to neck site, age and general condition of the patient needs to be differentiated from tumor induced patterns in future morphologic investigations of cervical lymph nodes in patients with head and neck carcinoma.

The cervical lymphatic system is part of a complex immune network. Over the last several decades, extensive research has demonstrated its sophisticated function as more than a simple lymphatic drainage pathway. Rather, it is a complex system of lymph nodes as organs of interaction between locally drained immune modulating substances (e.g., tumor-antigens, cytokines), on the one hand, and nodal as well as circulating immune competent cells, on the other hand.

Certain morphologic immune reaction patterns of regional lymph nodes have been reported as significant prognostic factors for survival of tumor patients with squamous carcinoma of the head and neck (1-5). Others, however, have failed to demonstrate such a correlation (6). Nonetheless, these morphologic immune reaction patterns are not restricted to tumor-draining lymph nodes (7). For this reason, it has been suggested that

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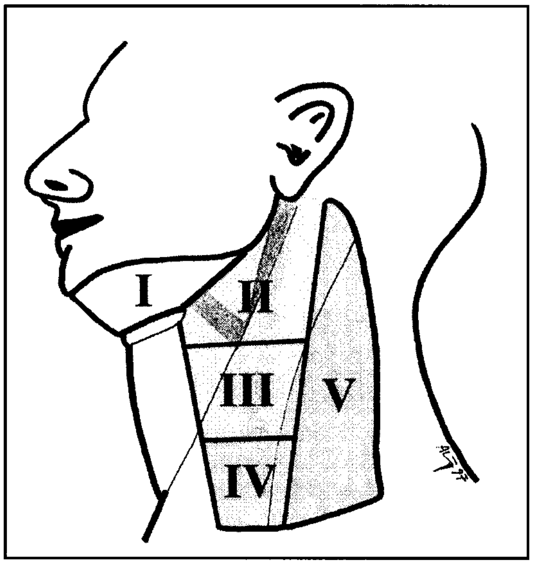


Fig. 1. Cervical lymph nodes grouped according to five defined neck levels (8).

the reported histologic features are likely non-specific for tumor-host immune response, and rather reflect the general condition of the subject (7). Due to this disparity, histomorphologic reaction patterns of regional host immune response have not thus far been established as an independent prognostic factor for patients with head and neck carcinoma.

Accordingly, differentiation between tumor related and tumor independent lymph node reactivity is crucial for further investigation of immunologic tumor-host interaction in lymph nodes regional to head and neck cancer, especially in those patients without metastatic disease. This distinction requires comprehensive insight into the immune reaction patterns of cervical lymph nodes as a function of their anatomical site as well as the age and general condition of the patient. Such detailed knowledge about the prevalence and the regional distribution of immune reaction patterns in cervical lymph nodes of individuals without head and neck cancer is, however, still lacking. Whereas several investigators have claimed that the site and incidence of cervical metastases depends on localization and stage of the primary tumor (8-10), the "normal" morphology of human cervical lymph nodes and its variants according to their anatomical site in the neck has attracted little or no attention.

In the present study the regional distribution of immune reaction patterns in cervical lymph nodes was investigated in cadavers at autopsy without disease of the head and neck region.

MATERIALS AND METHODS

Complete dissection of cervical lymph nodes from both sides of the neck was carried out in selected cadavers during standard autopsy in the Departments of Pathology and Forensic Medicine of the Rheinische Friedrich-Wilhelms-University (Bonn, FRG). Lymph nodes were grouped according to five defined neck levels (9): submental and submandibular triangle (Level I), upper (Level II), middle (Level III) and lower (Level IV) jugular chain and posterior cervical triangle (Level V) *(Fig. 1)*. An additional group of lymph nodes was removed from the tracheal bifurcation (thorax).

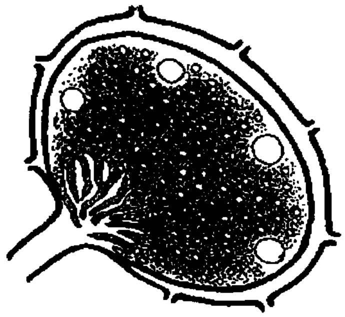
Only autopsied cases in excellent condition without evidence of relevant local or inflammatory diseases, sepsis or malignant tumors were included in the study. Cadavers with common chronic diseases, e.g., atherosclerosis or chronic obstructive lung disease (COLD) were not excluded. Routine work up of all autopsies was performed to investigate acute and chronic pathologic changes. Those showing advanced postmortem changes (autolysis) were excluded before or sometimes after histologic examination.

The neck specimens were fixed in 6% buffered formalin immediately after removal. Careful dissection of lymph nodes according to pathology standards, was performed before paraffin-embedding in detected lymph nodes in groups according to neck levels. In larger lymph nodes, a transverse section along the short axis of the nodes was done to assure

reaction pattern 1

”lymphocyte predominance“

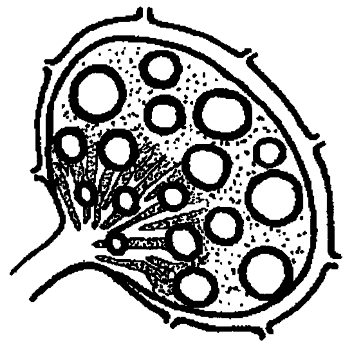
increased number of small lymphocytes in cortex, paracortex and medullary regions; expanded paracortex with prominent endothelial cells in capillaries and postcapillary venules.



reaction pattern 2

”germinal center predominance“

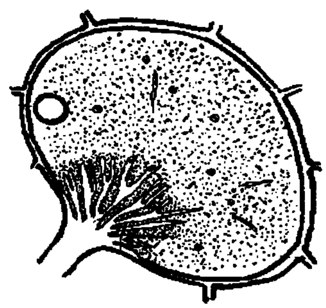
lymph follicles with germinal centers containing large lymphoid cells and mitotic figures; unremarkable paracortex; enlarged medullary cords with plasmoblasts and plasmocytes.



reaction pattern 3

”unstimulated node“

cortical lymphoid follicles without germinal centers; unremarkable paracortex; unremarkable medullary cords; no significant fibrosis or hyaline deposits.



reaction pattern 4

"lymphocyte depletion“

hypocellular cortex and paracortex; fibrosis; hyaline deposits in paracortex and medullary cords, occasionally extensive amounts of hyaline in any part of the node; absence of germinal centers.

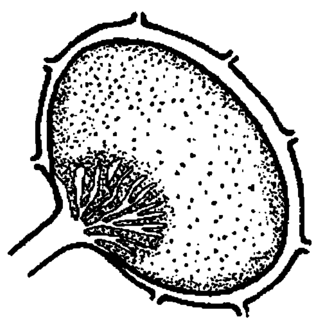


Fig. 2. Histomorphologic immune reaction patterns of lymph nodes according to Tsakraklides, et al (11).

that all important structures were represented in the histologic slides. For subsequent histologic examination, 4 µm thick sections were taken and stained with hematoxylin and eosin (H&E).

After complete routine-work up, sections of 1024 cervical lymph nodes in 242 groups of 50 neck dissection specimens from 25 selected cases formed the material for this study. Eleven patients had died because of acute drug intoxication or acute trauma without severe hemorrhage and autopsy showed no evidence of relevant chronic disease. Neck dissection specimens of these individuals were classified as "healthy". The remaining fourteen subjects displayed chronic diseases, especially from generalized atherosclerosis or chronic obstructive lung disease (COLD) and died as a consequence of complications of these disorders (e.g., stroke, acute myocardial infarction or respiratory insufficiency).

The lymph nodes were examined by light microscopy with special attention to lymph follicles, germinal centers, deep cortical regions, postcapillary venules, fibrosis, hyaline deposits, sinus histiocytosis and overall lymphocyte content. Based on these histomorphologic features, each group of nodes was classified into one of four distinct immune reaction patterns (RP) according to the criteria first described by Tsakraklides and coworkers (3,11) *(Fig. 2)*. Because the classification is based on distinct histomorphologic criteria, stratification was unambiguous in the vast majority of lymph nodes. Where lymph nodes with more than one reaction pattern in one single level was observed, the most frequent reaction pattern was assigned. Levels with no lymph nodes or only one lymph node less than 5 mm of size were excluded from further evaluation.

Statistical analysis of data was done based on neck sites (n=50) and lymph node groups (n=242), respectively. Analysis based on lymph node groups instead of single nodes was chosen to avoid overrepresentation of specimens containing larger numbers of lymph nodes. For analysis of contingence tables the X^2-test was applied. Quantitative data were analyzed using the U-test (Mann-Whitney-Wilcoxon). The levels of significance are given with each result.

RESULTS

153 (63.2%) of the evaluated 242 cervical lymph node groups were classified as unstimulated according to RP 3 whereas 35 groups (14.5%) showed RP 1, 29 groups (12.0%) showed RP 2 and 25 groups (10.3%) showed RP 4. In the 25 thoracic lymph node groups no RP 1 (0%), two RP 2 (8%), 18 RP 3 (72%) and 5 RP 4 (20%) were detected.

An overview of the regional distribution of immune reaction patterns according to neck levels is shown in *Fig. 3*. The histologic evaluation clearly demonstrated a higher incidence of RP 1 and RP 2 in level I (submandibular triangle) and level II (upper jugular chain). Level III showed intermediate stimulation patterns while RP 4 predominated in level IV (lower jugular chain) and level V (posterior triangle). These findings were highly significant in the X^2-test ($p<0.001$).

Age and general health status showed an influence on the detected reaction patterns. In level I and level II, a higher prevalence of immune stimulated lymph node groups (RP 1 or RP 2) was found in the group of "healthy" cadavers whereas regressive lymph node changes (RP 4) were only occasionally found in level IV of both groups but were more often detected in level V of cases with chronic diseases.

These findings were confirmed by statistical analysis. In neck specimens with one or more lymph node levels showing RP 2 (germinal center predominance), the mean age of subjects was 49.1±19.3 years (n=19 neck specimens) compared with 62.1±13.5 years in those without RP 2 (n=31 neck specimens; $p<0.05$). The incidence of RP 2 was significantly higher in the group of healthy subjects ($p<0.01$). In neck specimens with lymph node levels showing RP 1

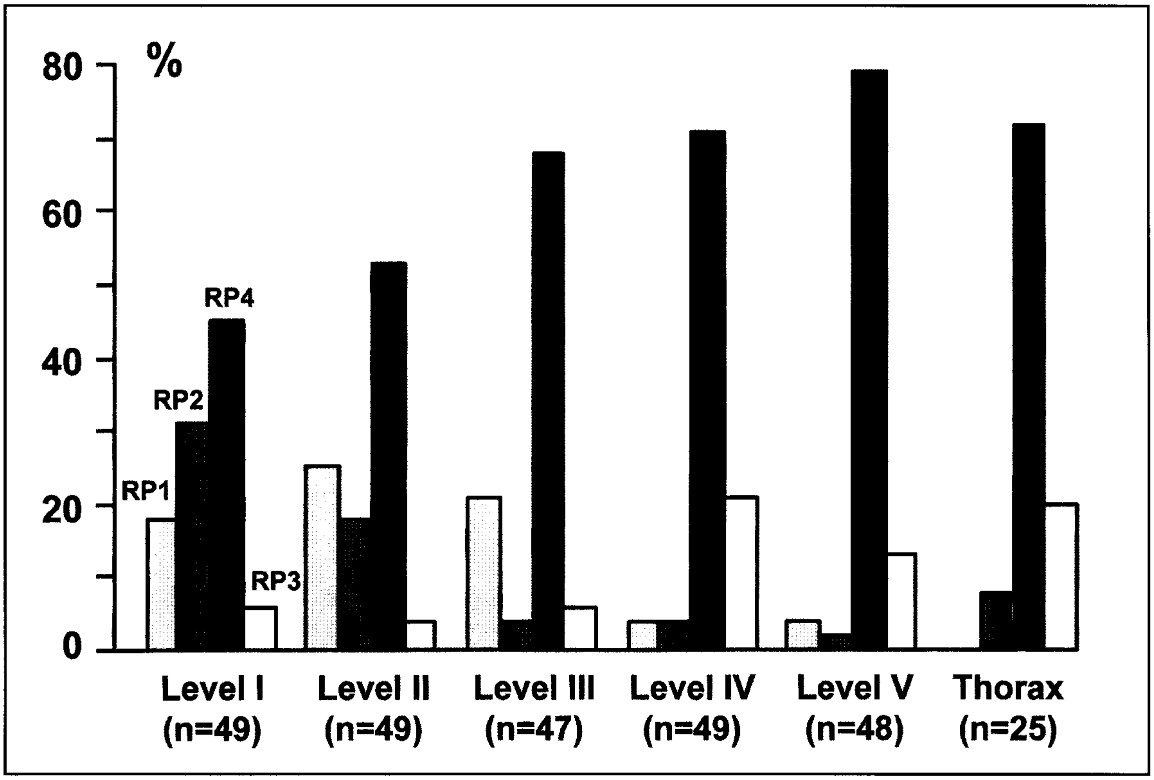


Fig. 3. Histomorphologic immune reaction patterns in cervical lymph node groups according to five defined neck levels (see Fig. 2) and also in lymph node groups adjacent to the tracheal bifurcation (thorax). RP 1 – lymphocyte predominance; RP 2 – germinal center predominance; RP 3 – unstimulated node; RP 4 – regressive changes.

(paracortical activation), the mean subject age was 54.3±16.2 years (n=19 neck specimens) compared with 58.8±17.6 years in those without RP 1 (n=31 neck specimens; N.S.). RP 1 also failed to show a significant correlation to general health status. In neck specimens with lymph node levels showing RP 4 (regressive changes), the mean age was 66.4±11.9 years (n=18 neck specimens) compared with 51.9±17.4 years in those without RP 4 (n=32 neck specimens; $p<0.05$). Occurrence of RP 4 was also significantly higher in subjects with chronic diseases ($p<0.01$).

DISCUSSION

In the investigated neck dissection specimens all types of lymph node immune reaction patterns could be detected. Although most of lymph node groups showed no essential signs of reactive changes, nearly one-third displayed patterns of lymphocyte predominance (activation of the paracortical zone), germinal center predominance or regressive changes. Therefore, we agree with Carter (7) that the four reaction patterns described by others (3,11,15) are not restricted to regional lymph nodes draining head and neck cancer and thus do not necessarily reflect a specific tumor-host interaction.

Our results also suggest a distinct distribution and pattern of morphologic immune reactions in cervical lymph nodes systematically related to their site in the neck.

This conclusion is consistent with previously reported findings in neck dissection specimens of patients with head and neck carcinoma (12). In the present investigation, the existence of immune stimulated lymph nodes showing lymphocyte predominance (RP 1) or germinal center predominance (RP 2) in the submandibular triangle (Level I) and the upper jugular region (Level II) independent of neoplastic diseases of the head and neck region has been demonstrated. The occurrence of immune stimulated lymph nodes in these neck levels underlies their involvement in regional immune response. This is most likely due to constant antigen exposure of the mouth and the oropharynx. Although we selected cadavers without evidence of relevant local disease, this does not necessarily exclude subtle inflammation such as subclinical chronic tonsillitis or solitary inflammed tooth roots. By contrast, in more distant lymph nodes from the lower jugular chain (Level IV) or the lateroposterior neck (Level V), more regressive changes and only occasionally immune stimulation could be detected. Those findings are also consistent with rare immunoreaction in the more remote thoracic lymph nodes. Taken together, our results support a hierarchical structure of the cervical lymphatic system previously proposed by Klimek and coworkers (12) parallel to the well known architecture of the cervical lymphatic drainage (13).

Individual age and general condition correlates with the occurrence of lymph nodes showing germinal center predominance (RP 2) or regressive changes (RP 4). This finding is consistent with those of other investigators examining human lymph nodes from different sites of the body (14,15) and possibly reflects an individual's general health status in relation to the cervical immune system. However, no such correlation was detected for the lymphocyte predominance reaction pattern (RP 1) which is thought to be the morphologic counterpart of increased T-cell activity (16).

Functionally, a hierarchical immune suppression in patients with squamous carcinomas of the head and neck has been proposed by Wang and coworkers (17). These investigators found lymphocytes from lymph nodes close to the tumor exhibiting a significant decrease in interleukin-2 activated cytotoxicity when compared to lymphocytes from distant nodes, an effect caused by tumor derived soluble suppressive factors (17,18). On the other hand, Schuller failed to demonstrate differences in tumor growth when incubating colonies of primary squamous cell carcinoma of the head and neck with lymphocytes derived from lymph nodes of different neck regions (19). It should therefore be emphasized that whereas the histomorphologic findings of lymphocyte or germinal center predominance in regional lymph nodes strongly suggest a hierarchical organization of the cervical lymphatic system, it does not follow necessarily in an individual patient that it reflect a positive immunologic host response.

In conclusion, tumor independent lymph node reactivity related to neck site, age and general condition of the patient must be differentiated from tumor induced patterns in future morphologic investigations of cervical lymph nodes in head and neck carcinoma.

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