Lymphocytic Infiltration in Undifferentiated Nasopharyngeal Cancer

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Background: Undifferentiated nasopharyngeal carcinoma (NPC) is characterized by prominent lymphocytic infiltration. Although the lymphoid infiltrate in NPC has been examined extensively in morphologic and immunocytochemical studies, the significance of this lymphoid infiltrate and its correlation with prognosis has been a subject of controversy for years.

Objective: To elucidate the significance of lymphoid infiltration in undifferentiated NPC.

Design: Evaluation of the relationship between lymphocytic infiltration in NPC and cervical lymph node status, ultrastructural examination of the lymphoid infiltrate, and assessment of lymphocytic infiltration as an independent prognosticator of regional node metastasis.

Materials and Methods: Lymphocytic infiltration was evaluated quantitatively in 20 cases of undifferentiated NPC using light microscopy. Four cases of undifferentiated NPC were processed for conventional electron microscopy. The effects of degree of lymphocytic infiltration, age, and tumor stage on cervical nodal metastasis were analysed using the logistic regression model.

Results: The degree of lymphoid infiltration correlated with cervical nodal metastasis ($P < .001$). Ultrastructural evidence of lymphocytes destroying cancer cells was seen. Lymphocytic infiltration was found to be an independent factor affecting cervical nodal metastasis ($P = .02$, univariate analysis; $P = .03$, multivariate analysis).

Conclusions: The lymphoid infiltrate is beneficial in undifferentiated NPC, and its presence may deter regional metastasis of cancer cells to the cervical nodes.


Nasopharyngeal carcinoma (NPC), one of the most common types of cancer in Southeast Asia, is a tumor of epithelial origin with a nonhomogenous architecture. The tumor has a multifactorial etiology and differs from other head and neck cancers by characteristic histological findings. It has been classified by the World Health Organization as including main types squamous cell carcinoma and nonkeratinizing and undifferentiated carcinoma.

The predominant type, undifferentiated carcinoma, has been described previously as a lymphoepithelioma. Based on the distribution of lymphocytes among the tumor cells, 2 patterns have been recognized, ie, the Regaud pattern, where the lymphocytes are present around the periphery of tumor cell aggregates, and the Schmincke pattern, where the lymphocytes are associated intimately within the tumor nests. The lymphocytic infiltrate is often accompanied by a reactive component consisting of eosinophils, dendritic cells, and macrophages. The prognostic significance of lymphocytic infiltration in undifferentiated NPC is controversial. On the one hand, the lymphoid infiltrate is thought to represent a local immune reaction against NPC and is associated with a good prognosis. Hang et al have demonstrated evidence of lymphocyte-mediated cancer cell lysis by the lymphoid infiltrate (which consists essentially of T lymphocytes). On the other hand, lymphocytic infiltration has also been reported to be unrelated to better survival in patients with NPC.

In this study, we investigated lymphoid infiltration in undifferentiated NPC under light and electron microscopy and analyzed the effect of the degree of lymphocytic infiltration with variables such as age and tumor stage on cervical node metastasis.

RESULTS

HISTOLOGICAL FINDINGS

Lymphocytic infiltration was present in all 20 cases of undifferentiated carcinoma. The tumor cells were arranged in irregular or moderately well-defined masses in a lymphoid stroma. The lymphocytes appeared as heavy hematoxylin-stained nu-
MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded tissue samples from 20 patients with undifferentiated NPC (World Health Organization type 3) before treatment were included for the light microscopic study. Eight patients with no cervical node metastasis met the American Joint Committee on Cancer11 staging criteria for stage II cancer, and the other 12 patients with cervical node enlargement were classified as having stage III and IV cancer. The postnasal biopsy tissue samples were obtained from 20 men (17 Chinese, 2 Malay, and 1 Thai; mean age, 47 years). The presenting symptoms of these patients were decreased hearing, nasal symptoms such as discharge and epistaxis, and respiratory symptoms such as blood-stained sputum. Twelve patients (60%) already had cervical lymph node enlargement at the time of diagnosis (Table 1).

HISTOLOGICAL FINDINGS

All the specimens were reviewed by two of us (W.-M.Y. and A.J.). The density of the infiltrating lymphocytes was quantitated by counting the number of cells distributed within the carcinomatous nests (and not at the interface or the periphery of the tumor tissue) in 10 randomly chosen medium power fields (original magnification × 160).

TRANSMISSION ELECTRON MICROSCOPY

For conventional electron microscopy, tissues from 4 cases of undifferentiated NPC were fixed in 3% glutaraldehyde and 2% paraformaldehyde before osmication in 2% osmium tetroxide as previously described.12 Postosmicated samples were dehydrated in an ascending series of ethanol and embedded in araldite (Araldite 502 Resin; Ted Pella Inc, Redding, Calif). Ultrathin sections were cut and mounted on polyvinyl formal resin–coated copper grids (Formvar; Shawinigan, London, England). Grids were stained with uranyl acetate and lead citrate before viewing in a transmission electron microscope (JEOL 1200 EM; JEOL, Tokyo, Japan).

STATISTICAL METHODS

Commercially available software (SPSS package; SPSS Inc, Chicago, Ill) was used to study the relationship between variables such as age, tumor stage, and lymphocytic infiltration. For tumor stage, T1 and T2 were taken as group 1 and T3 and T4 were taken as group 2. Sample means and SDs were compared by means of the t test. P<.05 was considered statistically significant.

170±32 cells per 10 mpf) was significantly higher than those with cervical node disease (mean±SE, 51±9 cells per 10 mpf) (Figure 2; P<.001). Univariate and multivariate analyses showed that age and tumor stage were not associated with nodal metastasis, but that only the lymphocytic infiltration was related independently to the cervical nodal metastasis (Table 2).

ULTRASTRUCTURAL ANALYSIS

Conventional transmission electron microscopy of undifferentiated NPC revealed that the tumor tissue was heavily infiltrated with the lymphocytes. These lymphocytes, seen in close association with the malignant epithelial cells, conjugated with the tumor cells and caused alterations in the tumor cell membrane (Figure 3) and degradation of the cell organelles, which led to lysis of the tumor cell (Figure 4). The lymphocytes maintained their cellular morphologic features. Cancer cells not conjugated by lymphocytes remained intact.

COMMENT

Nasopharyngeal carcinoma, a rare tumor in Western countries but common in Oriental populations, has a high rate of treatment failure because of its rapid growth and highly metastatic behavior. Various prognostic factors have been studied in NPC in attempts to modify and potentiate the efficacy of treatment regimens. Age, histological features, and interval to recurrence have been found to be independent prognostic factors for overall survival, but only histological features and presence of complications were significant factors for the length of time with no locore-
gional progression.\textsuperscript{14,15} Hence, the outcome of disease clearly depends on the histological features of the tumor.

Undifferentiated NPC consists of a malignant component of epithelial tumor cells and a distinct lymphoid infiltrate. Lymphocytic infiltration among tumor cells is found in many other human malignant neoplasms, including breast cancers, colon carcinoma, lung cancers, malignant melanoma, and germinomas; their presence is associated with a good prognosis.\textsuperscript{9} In rectal cancer, semiquantitative assessment of the infiltrating lymphocytes was correlated with survival and was found to have an independent effect.\textsuperscript{16} In contrast, TNM staging rather than lymphocytic infiltration was observed to be related to prognostication in gastric cancer.\textsuperscript{17} Lymphocytic subpopulations infiltrating the squamous cell cancers of the head and neck, including the nasopharynx, reportedly indicate a more favorable prognosis.\textsuperscript{18,19} Our present study shows that lymphocytic infiltration is an independent factor affecting nodal metastasis and that this is not influenced by the stage of the tumor or the age of the patients with NPC.

The lymphoid cells within nasopharyngeal tumors are known to be cytologically normal.\textsuperscript{20} Immunocytochemical characterization of lymphocytes in NPC has shown that the lymphoid stroma, consisting mainly of a high density of mature T cells, were composed of a mixture of the phenotypes for Leu 3 (helper/inducer), CD8 (cytotoxic/Suppressor), and the receptor for interleukin...
2 (activated T lymphocytes) phenotypes. Other studies have recognized B lymphocytes, which are present rarely in the tumor parenchyma, as being of the L-26 or CD45R subtype.

The importance of T cells in tumor immunity, as evidenced by the direct killing of the tumor cells mediated via activated lymphocytes, was demonstrated some 30 years ago. Infiltrating lymphocytes are believed to be a reflection of the host immune response. Marked lymphocytic infiltration has been postulated to prevent lymphatic metastasis. Studies by Vose et al have shown a higher frequency of cytotoxicity in the tumor-infiltrating lymphocytes of NPC than in kidney or lung tumors. A significant cytolytic potential is reported to be latently present among the T lymphocytes in NPC, and the viral antigens (Epstein-Barr virus) expressed by the tumors are thought to be the targets of the cytotoxic T lymphocytes. Morphologic evidence of lymphocyte-mediated cancer cell lysis has been documented previously, and tumor-infiltrating T lymphocytes are known to increase interferon-γ production. Furthermore, it has been reported that immunotherapy of patients with the use of tumor-infiltrating lymphocytes and interleukin 2 seems to be effective in some advanced cancers such as metastatic melanoma, renal cell carcinoma, and lung cancers. Our ultrastructural studies have also provided evidence of the cytotoxic nature of these lymphocytes, supporting the notion that lymphocytes in NPC are nonmalignant elements.

Thus, the abundant lymphoid infiltrate observed in NPC would play a significant role in the biology of this neoplasm. In fact, it would appear that the infiltrating lymphocytes may have a role in preventing regional metastasis, thereby affecting prognosis, as evidenced by the significant difference in the degree of lymphoid infiltration in patients with NPC and without cervical lymph node disease and by the independent effect of the lymphocytic infiltration on nodal metastasis.

**CONCLUSIONS**

Lymphocytic infiltration is a striking feature of undifferentiated NPC. Lymphocyte-mediated lysis of NPC cells is evident from the morphologic features observed ultrastructurally. Because of their cytotoxic effect on NPC cells and their independent effect on cervical node metastasis, the infiltrating lymphocytes would affect tumor growth, progression, and spread of this highly metastatic neoplasm significantly.

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