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T-cell Rich B-cell Cross-reacting Nasopharyngeal Carcinoma

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Immunohistochemical analysis was carried out to examine infiltration of lymphocytes in the tumor nests of nasopharyngeal carcinoma (NPC) using 38 biopsy cases obtained from southern China. These cases were divided into 3 groups according to their prominent pattern associated with the cell and the tissue differentiation using the World Health Organaization (WHO) classification as follows: (i) 6 cases of squamous cell carcinoma (16%), (ii) 25 cases of differentiated non-keratinizing carcinoma (66%), (ii) 7 cases of undifferentiated carcinoma (18%). All tumor tissues reacted diffusely with MB-1, however, these did not react with L26 (CD20), 4KB5 (CD45R), MT-1, and leukocyte common antigen (LCA). In the tumor nests, normal lymphocytes were observed in squamous cell carcinoma type (50%), differentiated non-keratinizing carcinoma type (72%), and undifferentiated carcinoma type (86%). Therefore, it is suggested that a large number of infiltrating non-neoplastic T-cells may be related to good prognosis. T-cell-rich B-cell lymphomas (TCRBCLs) are recently described as unusual non-Hodgkin's lymphomas associated with predominance of reactive T-cells. Therefore, the mechanism of T-cell lymphocyte infiltration adjacent to the tumor cell is considered that these T-cell infiltrates may be the passive response to cytokine secretion by the tumor cells.

Introduction

Nasopharyngeal carcinoma (NPC) was first described as a neoplasm occurring in the nasopharynx, characterized by anaplastic cells surrounded by prominent infiltration of lymphoid cells. This tumor has been known as lymphoepithelioma. The nuclear features of the carcinoma cells are distinctive with large nucleus and prominent nucleolus. The cytoplasmic features of the carcinoma cells are variable and the cellular borders are often indistinct due to fusion of cytoplasms. NPC has been confirmed as arising from squamous epithelium by electron microscopical and immunohistochemical examination. According to the World Health Organaization (WHO) classification, NPC was divided into three types; squamous cell carcinoma, differentiated non-keratinizing carcinoma, and undifferentiated carcinoma.[12] Lymphoepithelioma occurs outside of the nasopharynx, although generally they are rare lesions. Histologically similar undifferentiated carcinoma associated with marked lymphoid cell infiltration is called lymphoepithelioma-like carcinoma which has been found in the tonsil,[9] salivary gland,[2, 10] thymus,[16] uterine cervix,[5] skin,[15] lung,[1] and stomach.[14]

Normal small lymphocytes are frequently observed in tissue sections. They are often found in follicular lymphomas and also present in many diffuse lymphomas. It may be impossible to determine by routine histological techniques whether normal small lymphocytes are part of the neoplastic proliferation or they are reactive or residual benign cells. The large number of T-cells in T-cell-rich B-cell lymphomas (TCRBCLs) has been regarded as an active reaction to the neoplastic B-cells.[3, 4, 6, 8] It is suggested that T-cell infiltration may be a passive response to cytokine production by the tumor cells.

In this report, we described immunohistochemical features of T-cell lymphocyte infiltration in 38 cases of NPC biopsy obtained from southern China, and also discussed mechanism of normal T-cell lymphocyte infiltration adjacent to neoplastic cells.

Materials and Methods

The NPC specimens from 38 biopsy cases at the Jinan University Hospital in southern China were used. Shanmugaratnam and Sobin criteria were applied in assigning the diagnosis of NPC to the tissue. These NPC cases were divided into three groups as follows: (1) squamous cell carcinoma type, (2) differentiated non-keratinizing carcinoma type, and (3) undifferentiated carcinoma type.[12]

The specimens were fixed in 10% formalin, and embedded in paraffin for histological and immunohistochemical studies. Sections were cut at 4 micron and stained with hematoxylin and eosin stain for histological examination. These specimens were stained by indirect peroxidase-antiperoxidase (PAP) method for B-cells (monoclonal antibody; Bio-science products; Switzerland; anti-leukocyte B-cells (MB-1); Lot. 050), T-cells (monoclonal antibody; Bio-science products; Switzerland; anti-

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leukocyte T-cells (MT-1): Lot. 101), L26 (monoclonal antibody; Dako Corp., U. S. A., for identification of human B-cell lymphoma; CD20; Lot. 090), 4KB5 (monoclonal antibody; Dako corp., U. S. A., for identification of human B-cell; CD45R; Lot. 109), leukocyte common antigen (LCA) (monoclonal antibody; Dako Corp., U. S. A., PAP kit 0535, Lot. 00098), S-100 protein (polyclonal antibody; Dako Corp., U. S. A., PAP kit K0524, Lot. 128-1), lysozyme (polyclonal antibody; Dako Corp., U. S. A., PAP kit K504, Lat. 128-2), and alpha-1-antichymotrypsin (ACT) (polyclonal antibody; Dako Corp., U. S. A., PAP kit K0534, Lot. 078-2). LCA, S-100 protein, lysozyme, and ACT were not diluted. MB-1, and MT-1 were diluted 1:200 with phosphate buffered saline (PBS) pH 7.4. L26, and 4KB5 were diluted 1:50 with PBS pH 7.4. The indirect PAP method was performed for the staining of MB-1, MT-1, L26, and 4KB5 using Dako universal kit for monoclonal antibody (Dako Corp., PAP kit K0550, Lot. 128-2).

The steps involved in the immunoperoxidase procedure are as follows: (1) Deparaffinize and hydrate in distilled water. (2) Treat with hydrogen peroxidase for 5 minutes. (3) Wash in Tris buffer pH 7.4 using three cycle changes of 3 minutes each. (4) Treat with normal serum for 20 minutes. (5) Treat with tap off excess normal serum from sections. (6) Treat with primary antibody for 3 hours in room temperature. (7) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (8) Treat with second antibody for 40 minutes in room temperature. (9) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (10) Treat with PAP for 40 minutes in room temperature. (11) Wash in Tris buffer pH 7 4 using three cycle changes of 5 minutes. (12) Treat with 3, 3-diaminobenzidine tetrahydrochloride (DAB) solution with hydrogen peroxidase for 5 minutes. (13) Wash in running water. (14) Nuclei stain in Mayer's hematoxylin for 2 minutes. (15) Wash in running water. (16) Dehydrate, clear, and mount.

Results

The patients ranged in age from 21 to 80 years with a mean of 46 years. Twenty-six cases were males and 12 cases were females. These NPC were classified into three groups according to their predominant pattern with hematoxylin and eosin stain on the basis of light microscopy. The first group consisted of 6 cases of squamous cell carcinoma type. The second was made up of 25 cases of differentiated non-keratinizing carcinoma type, and the third included 7 cases of undifferentiated carcinoma type.

All tumor tissues reacted with MB-1 (Fig. 1), but did not react with L26 (CD20), and 4KB5 (CD45R) as B-cell markers. These neoplastic tissues did not react with MT-1, and LCA. On the other hand, MB-1, MT-1, LCA, and 4KB5 reacted with infiltration of lymphoid cells adjacent to tumor tissues. Thirty-eight cases of B-cell antigen marker expression in the NPC associated with high content of reactive T lymphocytes were observed (Fig. 2). In the tumor nests, T-cell lymphocytes were detected in squamous cell carcinoma type (50%), differentiated non-keratinizing carcinoma type (72%), and undifferentiated carcinoma type (86%). Therefore, there was a rich infiltrate of mature T lymphocytes that comprised more than 70% of the cellular population. These infiltrated cells did not react with S-100 protein, ACT, and lysozyme, thus, histiocytes and dendritic cells were not observed in NPC tissues.

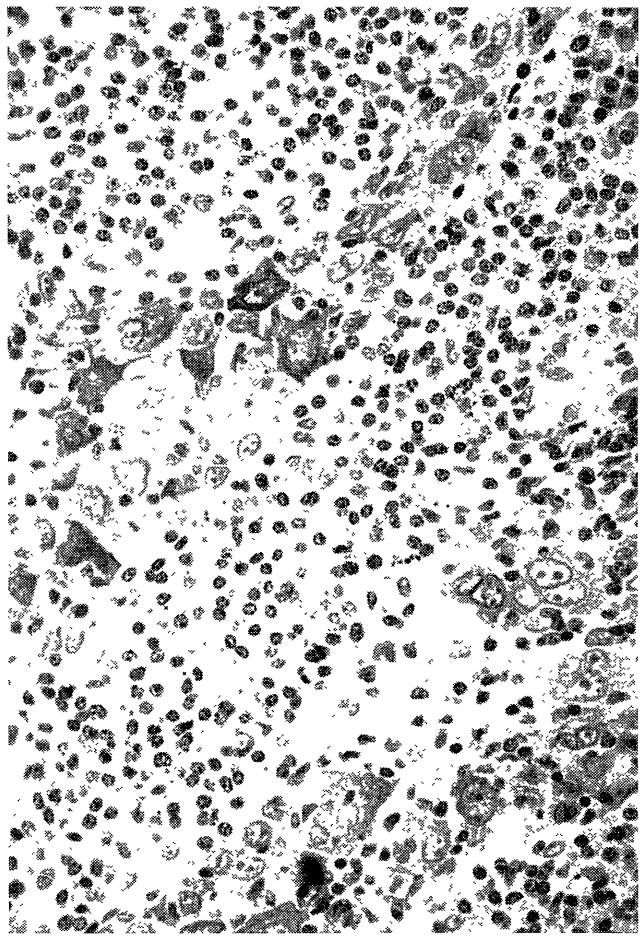

Fig. 1. Undifferentiated carcinoma type of nasopharyngeal carcinoma cells strongly reacted with MB-1. A few infiltrate lymphocytes were stained by MB-1, thus, most of normal lymphocytes were showed T-cell nature. Immunoreaction for MB-1, X 200.

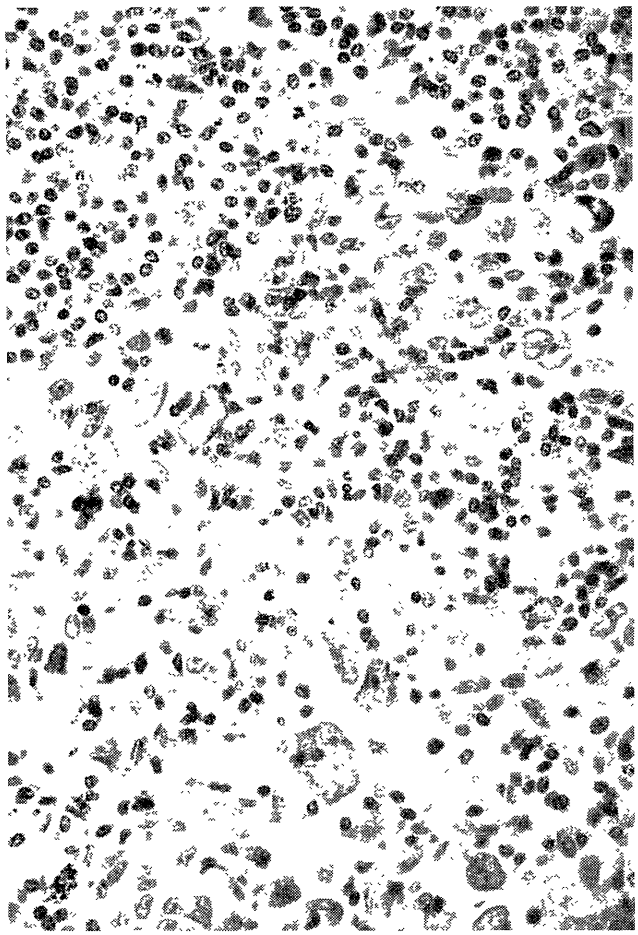

Fig. 2. Undifferentiated carcinoma type of nasopharyngeal carcinoma cells did not reacted with MT-1. Most of infiltrate lymphocytes were stained with MT-1. Immunoreaction for MT-1, X 200.

Discussion

Thirty-eight cases of NPC obtained from southern China were analyzed using immunohistochemical procedure employing various antibodies. All cases of NPC reacted with MB-1, thus, these cases were expressed as B-cell antigen marker in NPC tissues. The EBV is associated with a variety of benign and malignant lymphoproliferative disorders, most notably infectious B-cell lymphoma and NPC.[7,17] The mechanism of MB-1 positive tumor cells is not clear. A possible explanation may be that NPC produces one of B-cell antigen, or NPC has similar epitope such as reacted with MB-1. Malignant tumors primarily arising in the nasopharynx are most often carcinoma or malignant lymphoma. Undifferentiated NPC is accompanied by prominent lymphocytic infiltration, thus, it is often difficult to differentiate from malignant lymphomas, especially of the large-cell type. NPC is a definite malignant epithelial neoplasm and can be distinguished from malignant lymphoma by immunohistochemical staining for keratin, EMA, and LCA.[11,13,18]

The authors reported 38 cases of NPC which expressed B-cell antigen marker associated with abundant normal T-cell lymphocytes.[18] The mechanism of large number of normal T-cell lymphocytes detection in NPC is not clear, although, similar phenomenon is seen in TCRBCLs. In TCRBCLs, abundance of reactive T cells results in an appearance quite different from the classical descriptions of large B-cell lymphomas. TCRBCLs are not clearly designated in any non-Hodgkin's lymphoma classification. In the Kiel classification, most of the cases can be cassified cytologically as immunoblastic and centroblastic lymphoma. However, in the Working Formulation, it is uncertain whether they should be classified as mixed, small- and large-cell lymphoma, large-cell immunoblastic lymphoma, or unclassifiable lymphoma. TCRBCL cases resemble peripheral T-cell lymphomas (PTCLs), and are easily confused with PTCLs. Flow cytometry and frozen section immunoperoxidase methods in TCRBCLs may support the misdiagnosis of PTCL, because the large number of reactive T-cells may obscure the small B-cell population.[4] The exclusive use of frozen sections for immunohistochemistry increases the risk of such error, because morphological detail is poorly preserved and small neoplastic population may be hidden by large numbers of reactive T-cells, although, these disadvantages are avoided in paraffin sections. The B-cell nature of TCRBCLs is most readily recognized by paraffin immunohistochemistry of L26 which react with normal and neoplastic B-cell specificity of this new antibody.[4] The role of large number of T-cell lymphocytes in NPC is not clear. The possible explanation is that normal T-cell lymphocyte infiltration may be passive to cytokine secretion by the neoplastic cells. Therefore, morphological features of large number of T-cell lymphocytes present positive diagnosis of B-cell marker expressed neoplasms.

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