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Infiltrating Lymphocytes and Accessory Cells in Nasopharyngeal Carcinoma[1]

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The infiltrating lymphocytes (LCs) and accessory cells (ACs) including dendritic cells (DCs) and monocytes/macrophages in nasopharyngeal biopsies taken from 4 groups of nasopharyngeal carcinoma (NPC) patients were observed by using an immunostaining technique and the correlation of the results to the clinical manifestations and follow-up data was examined. The findings were as follows. (1) NPCs without lymph node metastasis always had marked infiltrating LCs and DCs as compared with those with lymph node(s) metastasis. (2) Advanced NPCs with lymph node(s) involvement (T1-4N1-3M0) and a rapid development of distant metastasis followed by death within 1 year after radiotherapy always showed fewer infiltrating LCs and DCs as compared with those with lymph node(s) metastasis (T1-4N1-3M0) and having longer than 5-year survival after radiotherapy. The amount of both LCs and ACs, especially DCs, infiltrating in NPC tissues appears to be an indicator of the activity of host immune defence mechanisms against cancer and influences the progression of the neoplasm as well as the prognosis.

Key words: Nasopharyngeal neoplasm — Lymphocyte — Accessory cell — Prognosis — Metastasis

Prominent lymphocytic infiltration in neoplastic growths, especially within carcinoma nests is a pathomorphological characteristic of nasopharyngeal carcinoma (NPC), which was formerly called lymphoepithelioma.[1)] Much work has been done to examine the significance of lymphocytic infiltration.[2-5)] Recently, with the advance of immunology, the role of accessory cells (ACs), including dentritic cells (DCs) and monocytes/macrophages (M/MCs), in NPC has attracted researchers' attention.[5)] We examined the biopsy slides of four groups of NPC patients by using an immunostaining method in order to evaluate the clinical significance of infiltrating lymphocytes (LCs) and ACs in NPC.

MATERIALS AND METHODS

Nasopharyngeal biopsies (1) Twenty-one early NPC cases without cervical lymph node metastasis and meeting the AJCC (American Joint Committee on Cancer) staging criteria[6)] (the recommendations of the AJCC in this revised manual and the publication of the UICC, in 1987, are identical) for stage I (T1N0M0) or stage II (T2N0M0), encountered in the Department of Pathology, Sun Yat-sen Memorial Hospital, in 1991 were chosen as Group I.

(2) Thirty-three advanced NPC cases with cervical lymph node(s) metastasis and meeting the AJCC criteria for stage III (T1-3N1M0) and stage IV (T4N1M0, T1-4N2-3M0), encountered in the Department of Pathology, Sun Yat-sen Memorial Hospital, in 1991 were chosen as Group II.

(3) Twenty-six advanced NPC cases with cervical lymph node(s) metastasis, meeting the AJCC criteria for stage III (T1-3N1M0) and stage IV (T4N1M0, T1-4N2-3M0) and showing a rapid development of distant organ metastasis followed by death within 1 year after redio-therapy in the Tumor Hospital, Sun Yat-sen University of Medical Sciences, over several years were chosen as Group III.

(4) Eighteen advanced NPC cases with cervical lymph node(s) metastasis, meeting the AJCC criteria for stage III (T1-3N1M0) and stage IV (T4N1M0, T1-4N2-3M0) and showing longer than 5-year survival after radiotherapy in the Tumor Hospital, Sun Yat-sen University of Medical Sciences, prior to 1986 were chosen as Group IV.

All the biopsy tissues were taken before radiotherapy and immediately fixed in 10% neutral formalin, and then paraffin-embedded. The paraffin blocks were taken from storage and re-sectioned (5 μm thickness) consecutively for hematoxylin and eosin (H&E) staining and immunostaining. Fresh biopsy tissues of Group I and II cases were also cryosectioned (5 μm thickness) for applying CD4 and CD8 immunostaining.

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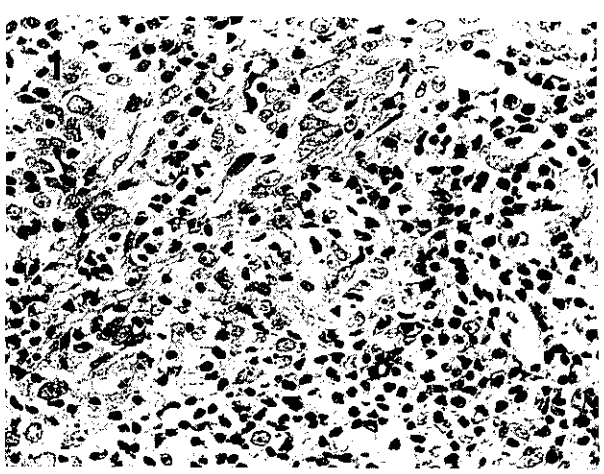
Immunostaining The general ABC procedure for use with polyclonal rabbit or monoclonal mouse primary antibodies was followed according to the DAKO Handbook.[7] The antibodies used in this study were all purchased from Dakopatts, Ltd., Copenhagen, Denmark (Table I).

Evaluating the presence and frequency of LCs, DCs and M/MCs in histological sections The criteria used in this study were essentially those of Giannini *et al.*[5] Infiltrating LC density was semiquantitatively evaluated by counting the number of cells distributed within carcinoma nests in 8–10 randomly chosen medium power ($\times 160$ magnification) fields. Three degrees of lymphocytic infiltration were identified: "Slight" ≤ 5 cells/field; "moderate" 6–20 cells/field; and "marked" ≥ 21 cells/field. The LCs were recognized not only depending upon H&E-stained sections but also upon supplementary immunostainings such as CD3, UCHL-1, L26 and CD45R. Five medium power ($\times 160$ magnification) fields were randomly chosen for counting the numbers of DCs and M/MCs. DCs were mainly distributed within the carcinoma nests, and only those DCs infiltrating within the carcinoma nests were counted. The density of DCs was evaluated as follows: "slight" ≤ 5 cells/field, "moderate" 6–10 cells/field; and "marked" ≥ 11 cells/field. M/MCs were mainly distributed surrounding the carcinoma nests, but in some cases, they were more often found within carcinoma nests. All the M/MCs either surrounding or within the carcinoma nests were counted. The density of M/MCs was classified as follows: "slight" ≤ 10

Table I. Antibodies Used

Code No.	Antibodies	Cells recognized	Working dilution	Paraffin (P) or frozen (F) section
A452	CD3	Pan-T cells	1:40	P
M472	UCHL-1(CD45R0)	T cells	1:50	P
M761	CD4	T cells helper/inducer	1:20	F
M707	CD8	T cells suppressor/cytotoxic	1:20	F
M731	IL2-R(CD25)	Activated lymphocytes	1:50	F
M755	L26(CD20)	B cells	1:50	P
M754	CD45R	B cells	1:30	P
Z311	S-100	DCs, etc.	1:300	P
A099	Lysozyme (muramidase) EC 3.2.1.17	M/MCs, etc.	1:300	P
A575	Keratin (human callus)	Carcinoma cells & epithelial cells	1:400	P



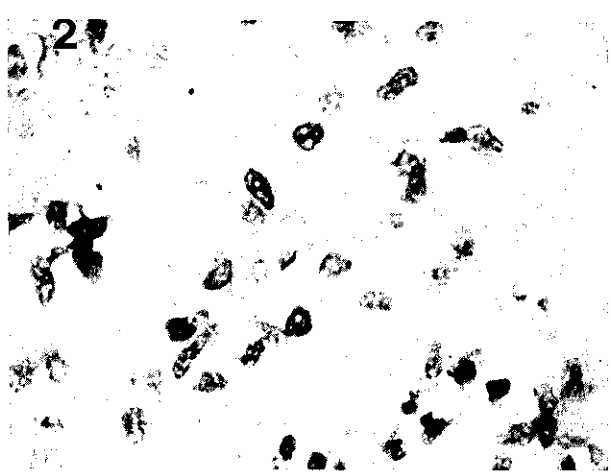


Fig. 1. Many lymphocytes infiltrating among carcinoma cells. H&E. Magnification $\times 200$.

Fig. 2. Infiltrating CD3-positive. Lymphocytes among carcinoma cells. CD3 immunostaining. Magnification $\times 200$.

cells/field; "moderate" 11–20 cells/field; and "marked" ≥21 cells/field. DCs could be recognized by their morphological aspect and S-100-positive reaction, and M/MCs were labeled by anti-lysozyme antibodies.

RESULTS

All the 98 nasopharyngeal neoplasms studied were diagnosed as non-keratinizing carcinoma or undifferentiated carcinoma according to the WHO classification.[1] The carcinoma cells reacted positively with anti-keratin antibodies, confirming that they were epithelial in nature.[8]

The LCs infiltrating within the carcinoma nests or among the carcinoma cells always appeared as heavy hematoxylin-stained nuclei with little cytoplasm on the H&E-stained sections (Fig. 1). Most of the infiltrating LCs belonged to the T-cell category, being CD3- and UCHL-1-positive (Fig. 2), while a few were of B-cell type, which could be recognized on L26- or CD45R-immunostained sections. The proportion of CD4+ cells to CD8+ cells varied from case to case; however, at least a portion of them could be classified as immunologically activated because they were interleukin-2-receptor (IL2-R)-positive.[9] The numbers of infiltrating LCs were counted according to the above-mentioned criteria and the results are summarized in Table II and Table III. Table II shows that there were statistically significant differences in between NPC cases of Group I and Group II when the LCs were "moderate" ($\chi^2=9.9926$, $P<0.005$) and "marked" ($\chi^2=17.8871$, $P<0.0001$). Table III demonstrates that there was a statistically significant difference between NPC cases of Group III and Group VI when the LCs were "slight" ($\chi^2=4.8589$, $P<0.05$), "moderate" ($\chi^2=5.1071$, $P<0.05$) or "marked" ($\chi^2=17.6282$, $P<0.0001$).

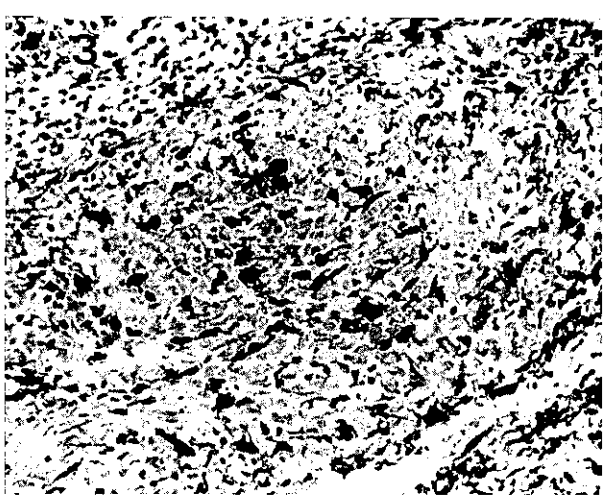
The vast majority of DCs was distributed among cancer cells (Fig. 3 and Fig. 4), but they were sometimes also found in the marginal zones between carcinoma nests and surrounding stromata. The findings of DC

Table II. The Numbers of LCs Found in NPC Cases of Group I and Group II

LCs	No. of cases (%)		χ^2 value	P value
	Group I	Group II		
Slight	2 (10.0)	9 (28.1)	1.5182	0.2179
Moderate	2 (10.0)	17 (53.1)	9.9226	0.0016
Marked	16 (80.0)	6 (18.8)	17.8871	<0.0001
Not assessable	1	1		
Total	21	33		

Table III. The Numbers of LCs Found in NPC Cases of Group III and Group IV

LCs	No. of cases (%)		χ^2 value	P value
	Group III	Group IV		
Slight	8 (30.8)	0 (0.0)	4.8589	0.0275
Moderate	13 (50.0)	3 (16.7)	5.1071	0.0238
Marked	5 (19.2)	15 (83.3)	17.6282	<0.0001
Total	26	18		



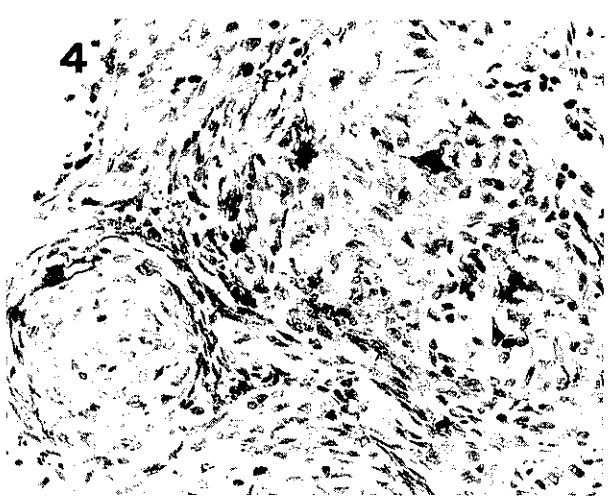


Fig. 3. Dendritic cells mainly infiltrating within carcinoma nests. S-100 immunostaining. Magnification ×174.

Fig. 4. Few dendritic cells could be found within carcinoma nests. S-100 immunostaining. Magnification ×200.

numbers in the 4 groups of NPC cases are shown in Table IV and Table V. Table IV shows that there was no statistically significant difference between NPC cases of Group I and Group II when the DCs were "slight" ($\chi^2=0.4750$, $P>0.05$), "moderate" ($\chi^2=2.4245$, $P>0.05$) or "marked" ($\chi^2=3.8289$, $P>0.05$). However, as can be seen in Table V, there were statistically significant differences between NPC cases of Group III and Group IV when the DCs were "slight" ($\chi^2=12.8365$, $P<0.0005$), and "marked" ($\chi^2=9.9000$, $P<0.005$).

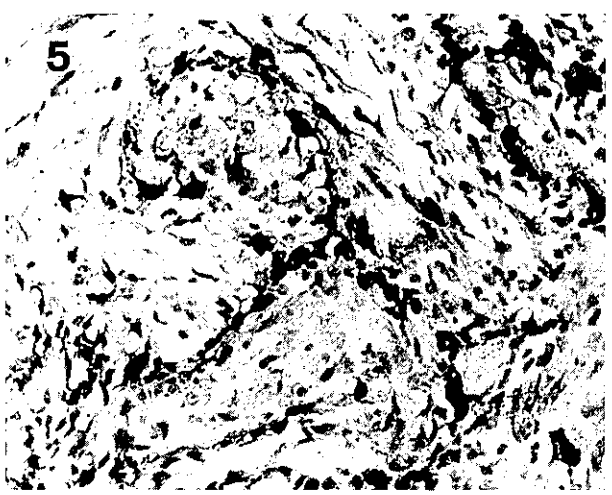
In contrast with DCs, the majority of M/MCs were mainly distributed in the marginal zones between carcinoma nests and surrounding stromata (Fig. 5 and Fig. 6), but they were sometimes found among cancer cells. The quantities of M/MCs in the 4 groups of NPC cases are shown in Table VI and Table VII. Table VI shows

Table IV. The Numbers of DCs Found in NPC Cases of Group I and Group II

DCs	No. of cases (%)		χ^2 value	P value
	Group I	Group II		
Slight	4 (21.1)	9 (30.0)	0.4750	0.4907
Moderate	3 (15.8)	11 (36.7)	2.4245	0.1195
Marked	12 (63.1)	10 (33.3)	3.8293	0.0504
Not assessable	2	3		
Total	21	33		

Table V. The Numbers of DCs Found in NPC Cases of Group III and Group IV

DCs	No. of cases (%)		χ^2 value	P value
	Group III	Group IV		
Slight	20 (77.0)	4 (22.2)	12.8365	0.0003
Moderate	3 (11.5)	4 (22.2)	0.2846	0.5937
Marked	3 (11.5)	10 (55.6)	9.9000	0.0017
Total	26	18		



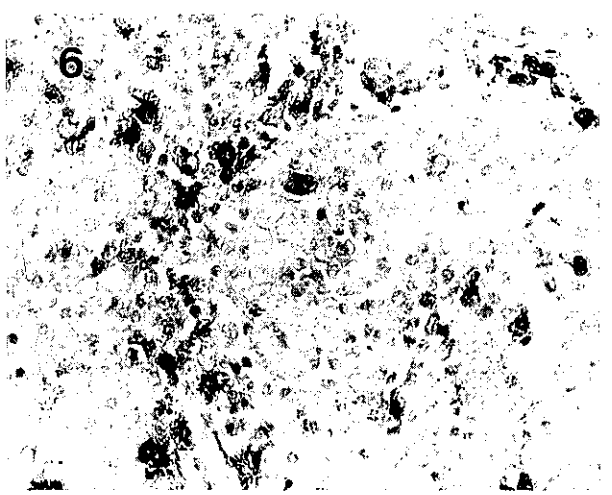


Fig. 5. A considerable number of monocytes/macrophages mainly infiltrating around the carcinoma cell clusters. Lysozyme immunostaining. Magnification ×200.

Fig. 6. Only a few monocytes/macrophages infiltrating in the tumor stroma. Lysozyme immunostaining. Magnification ×200.

Table VI. The Numbers of M/MCs Found in NPC Cases of Group I and Group II

M/MCs	No. of cases (%)		χ^2 value	P value
	Group I	Group II		
Slight	5 (26.4)	14 (46.7)	1.9499	0.1626
Moderate	7 (36.8)	5 (16.7)	1.5153	0.2883
Marked	7 (36.8)	11 (36.6)	0.0000	1.0000
Not assessable	2	3		
Total	21	33		

Table VII. The Numbers of M/MCs Found in NPC Cases of Group III and Group IV

M/MCs	No. of cases (%)		χ^2 value	P value
	Group III	Group IV		
Slight	16 (66.7)	2 (11.1)	11.1889	0.0008
Moderate	5 (20.8)	5 (27.8)	0.0896	0.7647
Marked	3 (12.5)	11 (61.1)	12.0485	0.0005
Not done	2	0		
Total	26	18		

that there was no statistically significant difference between NPC cases of Group I and Group II when the M/MCs were "slight" ($\chi^2=1.9499$, $P>0.05$), "moderate" ($\chi^2=1.5153$, $P>0.05$) or "marked" ($\chi^2=0.0000$, $P>0.05$). However, it can be seen in Table VII that there were statistically significant differences between NPC cases of Group III and Group IV when M/MCs were "slight" ($\chi^2=11.1889$, $P<0.001$), and "marked" ($\chi^2=12.0485$, $P<0.001$).

DISCUSSION

Lymphocytic infiltration can be found in many different kinds of tumors, and it is a pathomorphological feature of NPC. Infiltrating LCs in NPC have been the subject of previous investigations.[4, 5, 8] In this study, however, we were focusing mainly on the relationship between lymphocytic infiltration and the metastatic process. As shown in Table II, the amount of LCs infiltrating among tumor cells in Group I was greater than that in Group II. Since the critical difference between these two groups is whether the cervical lymph node(s) is involved, marked lymphocytic infiltration might reflect a beneficial immune response acting to prevent lymphatic metastasis. The infiltrating LCs were mainly T cells and therefore they may play a very important role in impeding cancer cell invasiveness and lymphatic metastasis. Among the T cells, both CD4+ and CD8+ cells could be demonstrated in cryosections by using CD4 and CD8 monoclonal antibodies, respectively. Both of them cooperate with accessory cells in the host immune response against neoplastic growth. However, the proportion of CD4+ and CD8+ cells fluctuated erratically from case to case, and there was no evident correlation to Groups I and II (results not shown). This is in agreement with Ferradini's finding.[10] It is noteworthy that only a portion of the T cells was IL2-R positive, and the other T cells were phenotypically different from IL2-R+ cells, presumably because impaired activation pathways are likely to develop in T LCs infiltrating NPC.[10] Table III shows another important finding, that is, the amount of infiltrating LCs in primary growths of advanced NPC cases (T1-4N1-3M0) seems to be an indicator for predicting whether hematogenous metastasis will develop. It seems that a larger amount of infiltrating LCs results in a lower frequency of hematogenous metastasis. This once again supports the conclusion that marked lymphocytic infiltration is a beneficial factor, impeding distant metastasis.

In recent years, the functions of ACs in the immune system have been extensively studied.[11] They include DCs and M/MCs, and are nonphagocytosing/phagocytosing, antigen-trapping, processing, and presenting cells. They also have other important functions. Early in 1985, Furukawa *et al.*[12] reported on the relationship between the degree of infiltration of DCs and M/MCs and the prognosis of lung cancer patients. As to NPC, however, only a few papers have been published,[5, 13, 14] and no concensus has emerged. Our observations confirmed that the DCs were mainly infiltrated within the carcinoma nests and M/MCs were distributed in the marginal zones between carcinoma nests and stromata. In some cases, M/MCs were found among cancer cells.[5, 13, 14] Table V shows that marked DC infiltration in tumor tissues of advanced NPC cases (T1-4N1-3M0) often (55.6%) implies a better prognosis ($P<0.005$) and slight DC infiltration frequently (77.0%) correlates with early distant metastasis and death ($P<0.0005$). Our data (results not shown) further indicated that the degree of DC infiltration was positively correlated with that of lymphocytic infiltration. Accordingly, the degree of DC infiltration as well as lymphocytic infiltration could influence the prognosis. This conclusion is consistent with the views of Nomori *et al.*[13] and Giannini *et al.*[5] However, Vera-Sempere *et al.*[14] concluded that the degree of DC infiltration in NPC had no prognostic significance. The discrepancy may be due to differences in the tumors examined (different combinations of patients with different clinical stages) and/or counting criteria adopted. Our study is still on-going.

As for M/MCs, there were statistically significant differences between Group III and Group IV. That is to say, the advanced NPC cases with longer than 5-year survival frequently (61.1%) had marked M/MC infiltration ($P<0.001$), whereas the rapidly progressive advanced NPC cases with early distant metastasis frequently (66.7%) had only slight M/MC infiltration ($P<0.001$). This result could be interpreted as indicating that M/MCs also influence the prognosis. Nomori *et al.*[13] indicated that M/MC infiltration was not related to prognosis, while Giannini *et al.*[5] claimed that moderate to marked M/MC infiltration resulted in a better survival rate. This issue needs to be studied further before a conclusion can be reached.

In summary, our results in this study suggest that LCs, DCs and M/MCs infiltrating NPC tissue are host immunity indicators which can influence the tumor progression and the prognosis, and these three kinds of immune cells appear to function coordinately in the defence mechanisms against cancer.

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