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Immunohistological characteristics of the infiltrating lymphoid cells and expression of HLA class I and II antigens in nasopharyngeal carcinoma

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Summary. The immunohistological characteristics of infiltrating lymphoid cells and the expression of human leucocyte antigens class I and II (HLA-ABC and HLA-DR, respectively) were studied in 50 pre-treatment nasopharyngeal carcinomas. The majority of lymphoid cells were activated lymphocytes expressing thymocyte OKT10 marker. CD4+ cells (T-helper/inducer) outnumbered CD8+ cells (T-suppressor/cytotoxic) by at least two- to four-fold. CD22+ cells (pan-B lymphocytes) were scanty in the peri-tumoral areas and were absent in 29 out of 50 biopsies. A moderate number of cells expressing CD15 (monocytes/macrophages) were also detected. CD16+ cells (natural killer cells) were found to be sparse or absent. Expression of HLA class I and II antigens on the tumor cells in 35 biopsies was variable. HLA-ABC staining was intense in 6, reduced in 13 and partially lost in 16, whereas staining of HLA-DR was intense in 7, reduced in 11 and partially lost in 17. Full expression of both antigens was demonstrable in only 2 biopsy samples. The expression of HLA antigens in the tumour had no relationship to the type or degree of lymphocytic infiltration or staging of the tumour.

Key words: Nasopharyngeal carcinoma – HLA antigens – Lymphocyte subsets

Introduction

Nasopharyngeal carcinoma (NPC) is rare in Caucasian populations but highly prevalent amongst Southeastern Chinese. Undifferentiated NPC, the commonest histopathological type in Southeastern Chinese, is aetiologically associated with Epstein-Barr virus (EBV) infection (Ho 1978). We have recently observed a stage-dependent lymphopenia in pre-treatment patients with NPC attributable to a decrease in pan-T (CD3+) and T-helper (CD4+) lymphocytes (Cheng et al. 1989).

Malignant tumours are often associated with marked mononuclear cell infiltration is suggestive of an immunological response developed by the host, analogous to the graft-versus-host reaction (Roitt 1988). Primary NPC has been reported to be infiltrated predominantly by T-lymphocytes (Wustrow et al. 1981; Thomas et al. 1984). Hence, a migration of T-lymphocytes from the vascular compartment into the diseased nasopharynx in response to tumour stimuli was suggested.

By analogy with organ transplantation, the human leucocyte antigens (HLA) of the major histocompatibility complex have been attributed an important role in tumour immunology (Roitt 1988). Anomalous expression of HLA antigens has been shown to correlate with the degree of differentiation and invasiveness of various malignancies (Natali et al. 1983; Darr and Fabre 1983; Momburg et al. 1986). In the present report, various lymphocyte markers and HLA class I and II antigens were studied in 50 NPC biopsies to determine the relationship between the degree and type of lymphocytic infiltration, the expression of HLA antigens in the tumour cells and the clinical staging of the disease.

Materials and methods

Nasopharyngeal biopsies from 50 Chinese patients (39 male and 11 female; mean age 44 years, range 26–79) with undifferentiated NPC were studied. The biopsy specimens were snap-frozen in liquid nitrogen and stored at −70° C. Six normal nasopharyngeal biopsies taken from patients with a negative EBV serology were used as controls. Fresh trophoblastic tissues and tonsils were similarly snap-frozen and used as positive controls. Clinical staging of tumour was based on the TNM classification according to Ho (1978).

The sources and reactivities of monoclonal antibodies used in this study are depicted in Table 1. The lymphocyte markers, HLA class I and II antigens expressed on cell surfaces were studied by the avidin-biotin-peroxidase complex technique modified from that reported by Hsu et al. (1983). No blocking of endogenous enzyme activities prior to incubation with monoclonal antibodies was made.

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Table 1. Immunohistochemical reagents used in the study of nasopharyngeal biopsies

Monoclonal antibodies	Predominant reactivity	Laboratory	Dilution
HLA-ABC	HLA class I antigen	Cappel, Turnhout, Belgium	1:100
HLA-DR	HLA class II antigen	Becton Dickinson, Mountain View, Calif.	1:200
Leu 2a (CD8)	T-(suppressor/cytotoxic) lymphocytes	Becton Dickinson	1:40
Leu 3a (CD4)	T-(helper/inducer) lymphocytes	Becton Dickinson	1:40
Leu 4 (CD3)	Pan-T lymphocytes	Becton Dickinson	1:40
Leu 11b (CD16)	Natural killer cells	Becton Dickinson	1:40
Leu 14 (CD22)	Pan-B lymphocytes	Becton Dickinson	1:40
Leu 17 (OKT10)	Thymocytes, activated T-cells	Becton Dickinson	1:40
Leu M1 (CD15)	Monocytes/granulocytes	Becton Dickinson	1:40

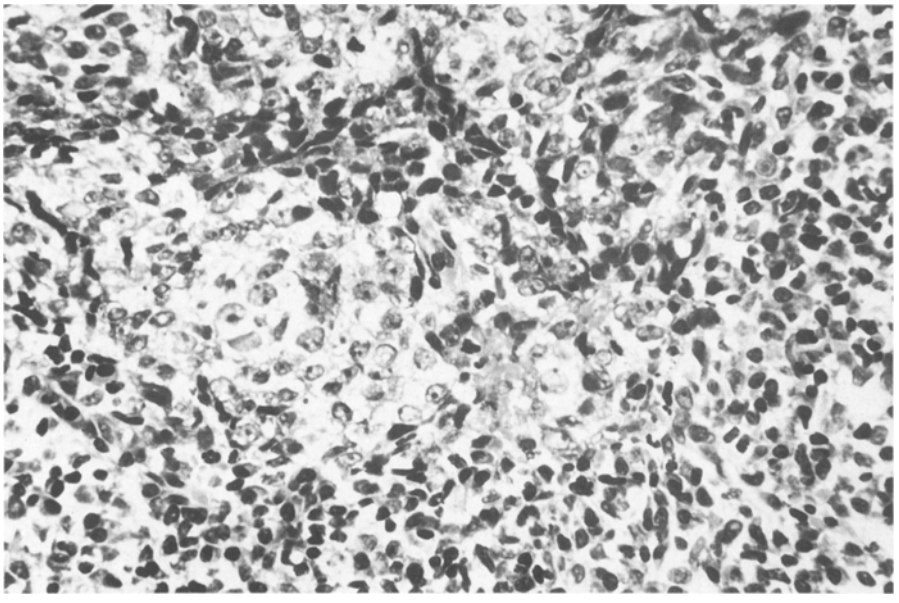

Fig. 1. Nasopharyngeal carcinoma exhibits heavy stromal infiltration by mononuclear cells. Haematoxylin and eosin, ×350

Positive staining was graded according to Momburg (1986) as follows: 1 = intense uniform staining of all tumour cells; 2 = reduced staining in all or some of the tumour cells; 3 = complete loss of staining in some of the tumour cells; and 4 = complete loss of staining in all the tumour cells.

Semi-quantitative grading of the infiltrating lymphocyte subsets was employed, where 0 = or less than 5%; 1+ = 5–20%; 2+ = 20–40%; 3+ = 40–80%; 4+ = 80–100%. Immunohistological assessment of tumour and infiltrating lymphocytes was made independently by two of the authors (PC and FML) with a concordance of 95% between observers.

Results

In all the biopsies, the tumour cells were large and exhibited a vesicular nucleus and prominent nucleoli with high nuclear cytoplasmic ratio. They formed cohesive sheets often with a syncytial appearance and were surrounded by dense lymphocytic infiltration (Fig. 1). Control positive stains for lymphocytes markers and HLA-DR antigens were confirmed in tonsils and negative staining was observed when primary antibody was omitted. For HLA-ABC antigens, villous trophoblastic cells showed no staining while other nucleated cells gave a uniform positive staining. Normal nasopharyngeal epithelium in 6 patients expressed both HLA-ABC and HLA-DR uniformly with an intensity of 2+ and 3+ respectively on a scale of 0 to 4+. The stromal mononuclear cells in these nasopharyngeal biopsies expressed CD3+ (75%–100%), OKT10 (50%–75%), CD4+ (50%–75%), CD8+ (25%–50%), while cells expressing Leu M1, CD22 and CD16 were usually small and scored 0 (sparse to less than 5%).

The nature of the lymphocytic infiltrations is presented in Table 2; they consisted almost exclusively of T-cells (CD3+). In 47 of the 50 biopsies (94%), most cells were activated T-lymphocytes with intense staining of OKT10 antigen (Leu 17). Heavy T-helper lymphocyte (CD4+) infiltrations (3+ or 4+) were present in 58% cases (29/50), while heavy infiltration by T-suppressor cells (CD8+) was observed in only 10 specimens (20%). T-helper cells in all but 7 cases (84%), exceeded T-suppressor cells by at least two- to four-fold (Fig. 2a, b). However, there was no direct correlation between the percentages of different lymphocyte subsets, CD4+/CD8+ ratios and the clinical staging of the disease. B-lymphocytes (CD22+) were scanty, generally less than 5% and were very scanty in 27 cases (54%). The small

Table 2. Composition of the peri-tumour cellular infiltrates and expression of HLA antigens in nasopharyngeal biopsies

No.	Sex/age	Stage[a]	CD8	CD4	CD3	CD22	CD4/CD8	OKT10	Leu M1	CD16	HLA[b]	
											ABC	DR
1	M/26	I	1+	3+	3+	0	6	2+	2+	2+	2	1
2	F/55	I	1+	1+	2+	0	1	3+	2+	1+	3	3
3	M/60	I	1+	2+	2+	1+	2	2+	2+	1+	ND	ND
4	F/37	II	2+	4+	4+	1+	2	3+	1+	0	2	3
5	M/34	II	3+	3+	4+	1+	1	4+	1+	1+	2	3
6	F/79	II	2+	3+	3+	1+	1	3+	1+	0	3	2
7	M/48	II	1+	4+	4+	0	4	3+	0	0	3	3
8	M/32	II	2+	4+	4+	1+	2	3+	1+	1+	ND	ND
9	M/36	II	1+	2+	3+	0	3	2+	1+	0	3	2
10	M/34	II	3+	3+	4+	1+	1	2+	2+	0	3	2
11	M/48	II	1+	1+	1+	0	2	3+	2+	0	3	1
12	M/36	II	1+	1+	1+	0	1	1+	1+	0	ND	ND
13	M/39	II	0	1+	2+	0	3	3+	2+	0	2	3
14	M/51	II	3+	3+	4+	0	1	4+	2+	0	2	3
15	M/28	II	1+	3+	3+	1+	4	3+	0	0	ND	ND
16	M/48	II	0	1+	1+	0	2	1+	0	0	3	1
17	M/34	III	2+	4+	4+	0	3	2+	2+	0	2	3
18	M/49	III	1+	2+	2+	1+	2	3+	2+	0	ND	ND
19	M/42	III	3+	4+	4+	1+	1	4+	2+	0	3	2
20	M/52	III	2+	3+	4+	1+	1	3+	2+	0	ND	ND
21	F/32	III	2+	3+	3+	1+	2	4+	1+	0	ND	ND
22	M/39	III	3+	4+	4+	2+	1	3+	2+	0	2	1
23	M/36	III	2+	4+	4+	1+	1	3+	1+	0	ND	ND
24	M/38	III	1+	2+	3+	0	2	2+	0	0	1	1
25	M/54	III	2+	2+	2+	1+	1	4+	1+	0	ND	ND
26	F/61	III	1+	2+	4+	0	4	3+	1+	0	3	3
27	F/35	III	0	4+	4+	0	10	3+	1+	0	2	2
28	M/41	III	2+	4+	4+	0	3	2+	0	0	ND	ND
29	M/45	III	1+	2+	3+	0	2	4+	0	0	1	2
30	F/57	III	3+	4+	4+	3+	1	2+	0	0	3	1
31	M/34	III	1+	4+	4+	1+	6	3+	1+	0	2	3
32	M/49	III	1+	2+	3+	0	4	4+	1+	0	3	3
33	M/43	III	2+	3+	4+	2+	2	3+	3+	0	ND	ND
34	M/39	III	1+	2+	4+	0	2	3+	1+	0	3	3
35	M/49	III	1+	4+	4+	1+	4	4+	0	0	2	3
36	M/66	III	2+	3+	4+	0	1	4+	1+	0	1	3
37	F/28	III	2+	2+	3+	0	1	3+	1+	0	2	3
38	F/58	III	0	0	2+	0	1	3+	2+	0	2	2
39	M/50	III	2+	3+	4+	0	1	4+	1+	0	ND	ND
40	F/40	III	3+	4+	4+	2+	1	4+	1+	0	ND	ND
41	M/62	IV	1+	2+	3+	1+	1	3+	0	0	ND	ND
42	M/50	IV	4+	4+	4+	0	1	4+	1+	0	3	2
43	M/56	IV	3+	4+	4+	0	1	4+	1+	0	2	2
44	M/62	IV	1+	1+	1+	1+	2	1+	1+	0	3	3
45	M/46	IV	2+	2+	4+	1+	1	1+	1+	0	ND	ND
46	M/32	IV	3+	4+	4+	1+	1	4+	2+	1+	3	2
47	F/69	IV	2+	2+	2+	0	2	2+	1+	1+	1	2
48	M/41	IV	1+	1+	1+	0	1	1+	0	0	3	3
49	M/52	IV	1+	3+	3+	0	2	3+	0	0	1	3
50	M/60	V	1+	4+	4+	0	6	4+	1+	0	1	1

ND, not done.
Semiquantitative assessment of lymphocytic infiltration in the peritumour areas:
0 = less than 5%; 1+ = 5–20%; 2+ = 20–40%; 3+ = 40–80%; 4+ = more than 80%.
[a] Ho (1978)
[b] Degree of expression of HLA-ABC (class I) and HLA-DR (class II) antigens in the tumour.
1, intense; 2, reduced; 3, partial loss; 4, complete loss

numbers of mononuclear cells in the peri-tumour infiltrates were macrophages/monocytes expressing Leu M1 marker (CD15). Natural killer cells (CD 16) were sparse and were absent in 50% of the cases.

The HLA class I and II antigens are expressed on both cell membrane and cytoplasm. Only 6 of the 35 specimens expressed HLA-ABC antigen fully (Table 3). Thirteen showed a reduced staining intensity in all or

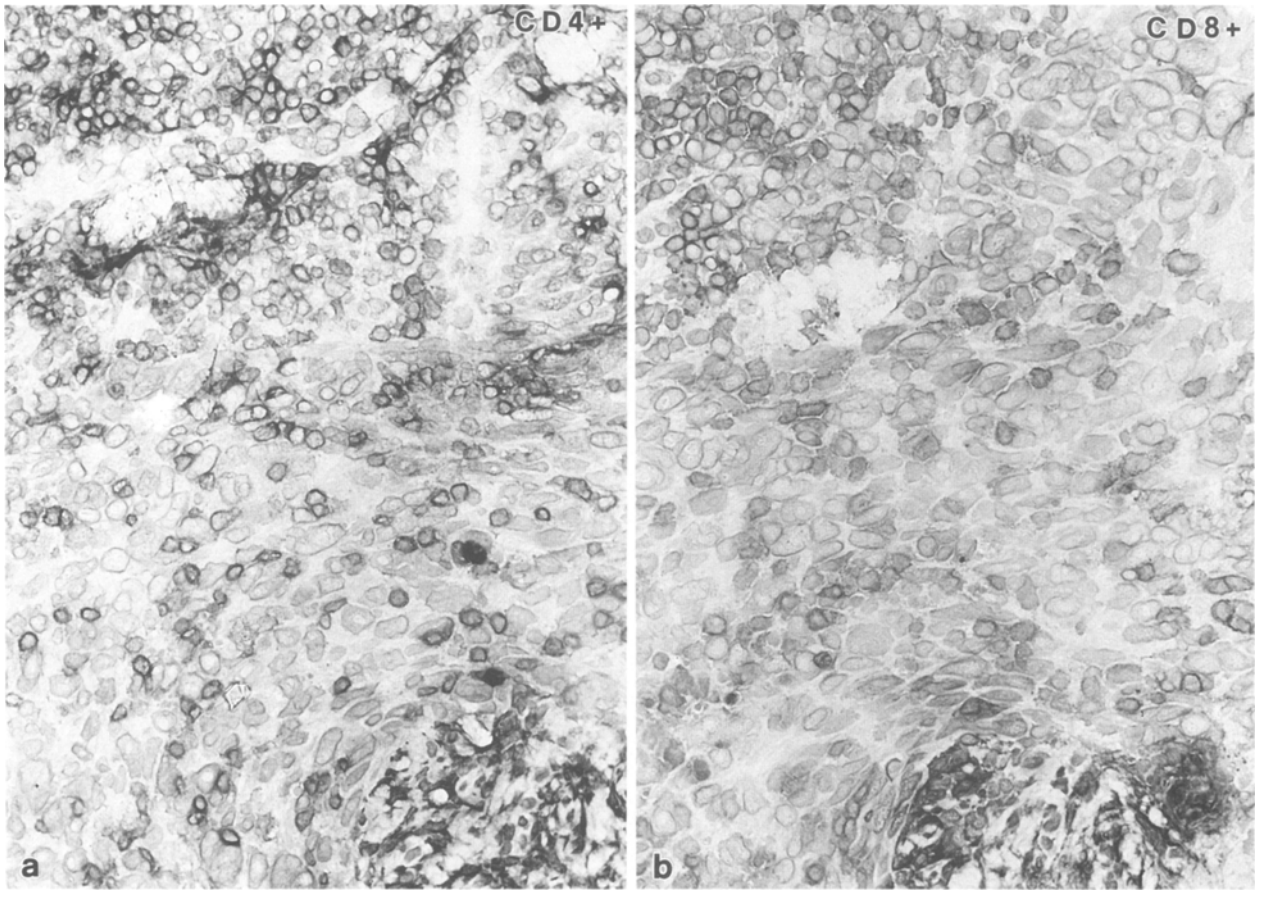

Fig. 2. a, b. T-lymphocyte subsets infiltrating stroma of nasopharyngeal carcinoma. **a** CD4+ lymphocytes (T-helper cells) are two- to four-fold heavier than **b** CD8+ lymphocytes (T-suppressor/cytotoxic cells). ABC immunoperoxidase method, ×680

Table 3. Number of tumour biopsies expressing HLA-ABC and HLA-DR antigens

HLA-ABC	HLA-DR			
	1	2	3	4
1	2	2	2	0
2	2	3	8	0
3	3	6	7	0
4	0	0	0	0

Semi-quantitative assessment of staining of HLA antigens: 1, intense; 2, reduced; 3, partial; 4, complete loss. Contingency table: chi-square test = 1.98, not significant, P more than 0.05

some of the tumour cells (Fig. 3a). Partial loss of staining was observed in 16 biopsies. Expression of HLA-DR was full in 7 specimens, reduced in 11 cases and partially lost in 17. No tumour was found to have lost completely either the HLA-ABC or HLA-DR antigens. Only 2 out of 35 biopsies examined fully expressed both HLA-ABC and HLA-DR. Seven biopsies had partial loss of both HLA-ABC and HLA-DR. The reduction in HLA-ABC antigen did not appear to correlate with the reduced expression of HLA-DR antigen (Table 3).

The intensity of HLA-ABC or HLA-DR staining did not correlate with the degree or type of lymphocytic infiltration or clinical staging of the tumour.

Discussion

In the present study, the characterization of the infiltrating lymphoid cells and the expression of HLA antigens were studied in 50 undifferentiated using a sensitive avidin-biotin immunoperoxidase complex technique. The lymphocytic infiltrates associated with NPC consist predominantly of CD3+ T-lymphocytes, a finding in keeping with previous reports among other ethnic groups using different methods (Jondal and Klein 1975; Wustrow et al. 1981). We have found that most CD3+ T-lymphocytes also expressed the OKT10 marker, indicating that they are activated or immunologically "switched on" as reported by Thomas et al. (1984). The majority of these T-lymphocytes are T-helper cells and only 20% of biopsy specimens demonstrate a predominant T-suppressor cell infiltration. The T-helper cells outnumbered T-suppressor cells by at least two- to four-fold in most cases. In contrast with the report by Wustrow et al. (1981), we found that B-lymphocytes (CD22+) accounted for not more than 5% of the total cellular infil-

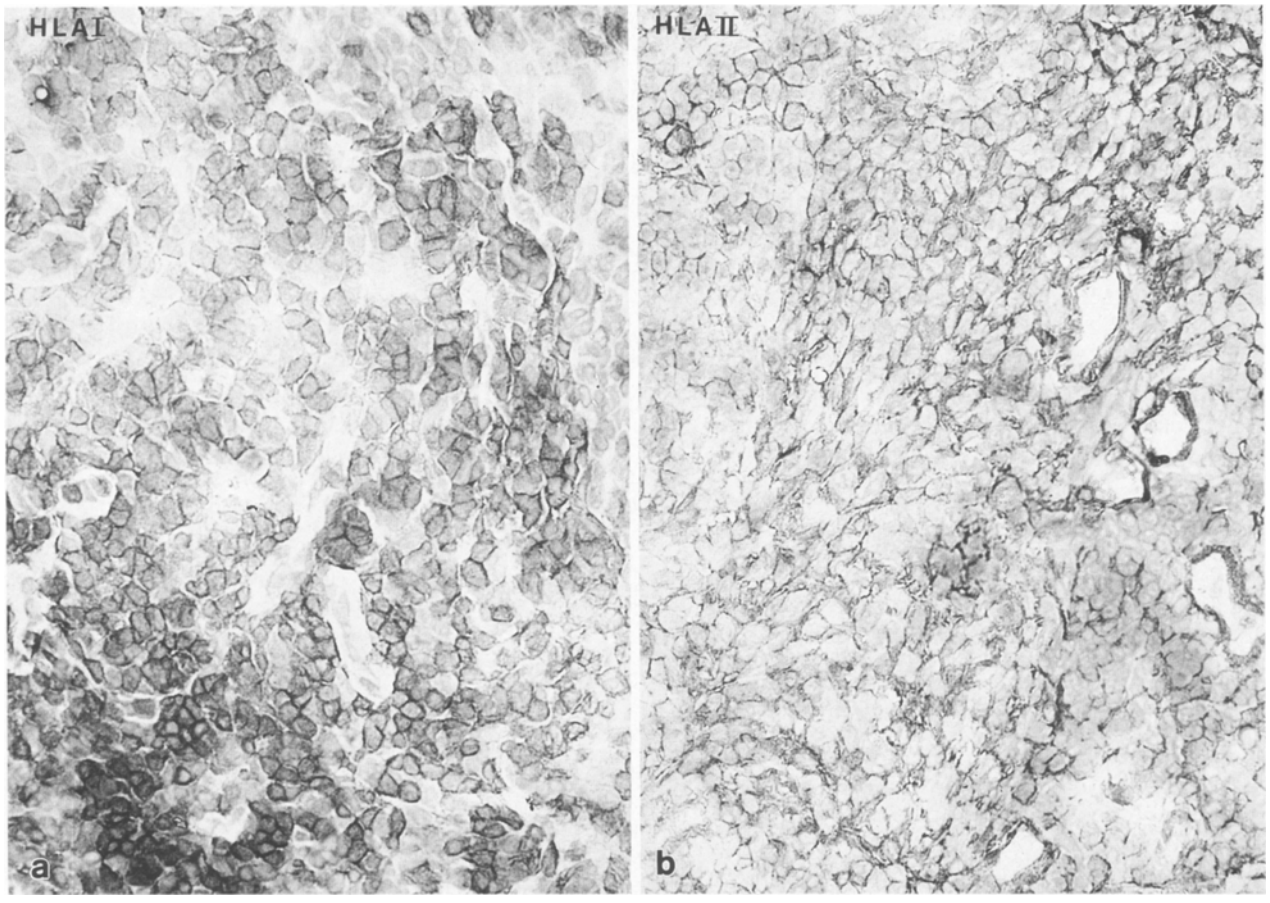

Fig. 3a, b. Undifferentiated nasopharyngeal carcinoma: **a** most tumour cells demonstrate membrane staining for HLA-ABC antigens, but a few have completely lost their expression. **b** Expression of HLA-DR antigens in many tumour cells; others show absence of staining. ABC immunoperoxidase method, ×680

tration and in 17 biopsies B-lymphocytes were absent. Furthermore, the paucity of natural killer cells (CD16+) and macrophages (CD15+) indicates that natural immunity plays a relatively minor role in NPC.

Other than an increase in T-helper cells, the lymphocytic infiltrates in NPC revealed no significant difference compared with normal control nasopharyngeal biopsies. It is intriguing that the host could not mount an effective anti-tumour response in the presence of activated T-lymphocytes. No significant correlation between different T-lymphocytes subsets in the tumour and their clinical staging was found.

HLA restriction is a prerequisite for recognition by effector T-lymphocytes and alteration in HLA antigens expression by malignant cells will compromise this recognition and may modify host defence. Decreased or loss of expression of class I or II antigens has been reported in various tumours (Ferguson et al. 1985; Natali 1983) and this could contribute to the failure of the hosts' recognition of the tumour and hence the lack of appropriate cellular immune response. The decrease in HLA antigen expression correlates with the aggressiveness of the tumours and with a shorter survival (Momburg et al. 1986; Spier et al. 1988). The expression of HLA antigens in normal nasopharyngeal mucosa is not known and we have shown that both HLA-ABC and HLA-DR antigens are uniformly present, although HLA antigen expression can be modified by viral infection (Grand et al. 1987). Our control biopsies from EBV-negative patients probably reflect the normal expression of HLA antigens. Both HLA-ABC and HLA-DR antigens were found to be reduced or partially lost in most of the 35 NPC specimens we examined. One would expect that these findings could affect the lymphocytic infiltration with altered tumour recognition. Nevertheless, no correlation between HLA antigen expression on tumour cells and T-cell subpopulation or clinical staging can be demonstrated. We have previously demonstrated a stage-dependent peripheral blood T-lymphopenia in pre-treatment NPC patients and have suggested that the T-cells in the tumour microenvironment could be sequestrated lymphocytes from the vascular compartment (Cheng et al. 1988). Furthermore, lymphocytes migrate selectively to lymph nodes in the neck containing metastases and perhaps to the primary NPC (Cheng et al. 1988). The present study thus further supports the notion that T-helper lymphocytes migrate to the diseased nasopharynx. Selective sequestration of T-lymphocytes reflects a cellular immune response despite a down-regulation of HLA antigen expression and suggests that tumour factors other than HLA antigens may be involved in the anti-tumour immune response.

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