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Immunohistological analysis of tumour infiltrating lymphocytes in seminoma using monoclonal antibodies

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Summary. Immunological characterization of tumour infiltrating lymphocytes (TIL) by immunohistological techniques was carried out in 20 cases of stage I seminoma. Routine pathological examination of these surgical specimens showed typical seminoma in 20 cases. Eighteen cases showed obvious TIL and immunohistological staining on frozen specimens was performed in 12. TIL in seminomas were predominantly T-cells but B-cells were also identified. T-cells were distributed diffusely with predominance of the CD 8+ phenotype judged semiquantitatively. In contrast to the distribution of T-cells, B-cells tended to accumulate and occasionally formed lymphoid follicles. In such follicles the phenotypic pattern of B-cell antigens was comparable with secondary lymphoid follicles in lymphoid organs. There is an immunologically complex response to seminoma by the host with a predominant infiltration of cytotoxic/suppressor T-cells and functional maturation of B-cells.

Key words: Seminoma – Immunohistological analysis – Tumour infiltrating lymphocytes

Introduction

Immunological reactions between cancer and host are believed to play an important role in regulating tumour cell growth. Lymphocytes found in tumour tissues, tumour infiltrating lymphocytes (TIL), are believed to exert these functions. Identification of TIL is clinically important because the degree of TIL is closely related to the prognosis of disease in some cancers (Ioachim 1976).

In the urogenital field the presence of TIL in seminomas is a very common feature (Thackray and Crane 1976) and is associated with an improved clinical survival (Mostofi and Sesternhenn 1976). However, only a few reports are available which describe the functional characterization of TIL in seminoma (Bell et al. 1987). In addition, no precise analysis of B-cells has been made in the TIL found in seminoma, although formation of lymphoid follicles has been described by routine histological examination (Thackray and Crane 1976).

The present study was undertaken in order to characterize the TIL in seminoma with a variety of monoclonal antibodies which identify functional subsets both for T-cells and B-cells.

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Materials and methods

Twenty patients with seminoma in clinical stage I were analysed in this study. The age distribution of the patients was between 23 and 60 years old, the average age being almost 35 years. Pathological examination was done on 5–10 sections from each surgical specimens using haematoxylin and eosin (H&E) staining. All 20 cases showed typical seminoma histologically.

After routine examination surgical specimens were immediately frozen in OCT compound (Miles) and stored at −80° C for immunohistological staining. Frozen sections made by cryostat (Miles) were dried in air and were then fixed in ice-cold acetone and indirect immunoperoxidase staining was performed. The monoclonal antibodies used in this study are listed in Table 1.

Monoclonal antibodies at appropriate dilution were applied on sections for 30 min at room temperature and the slides were washed with phosphate buffered saline. Then, horseradish-peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako, Kyoto, Japan)

Table 1. Monoclonal antibodies used in this study

Cell type	CD	Antibody	Reference
Pan-T	CD5	L1	SMC
	CD3	Leu4	BD
Suppressor/cytotoxic T-cells	CD8	L2	SMC
Helper/inducer T-cells	CD4	L3	SMC
Pan-B	CD2	L26	SMC
B-subset		L22	SMC

SMC, Sapporo Medical College, Takami et al. (1985); BD, Becton-Dickinson, Ledbetter et al. (1981)

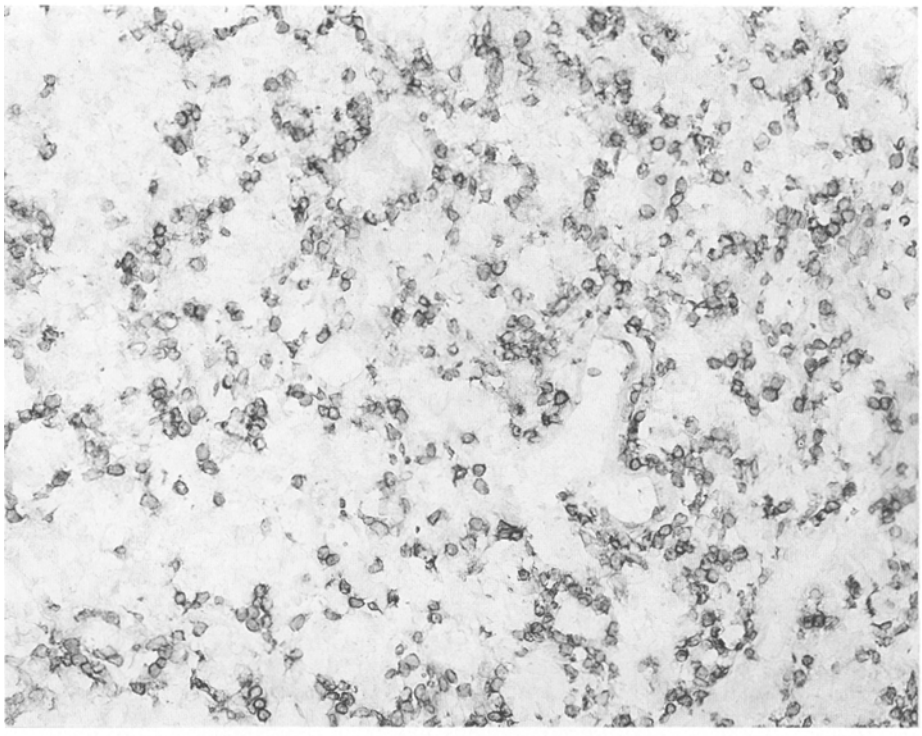

Fig. 1. L1 (CD5) staining on frozen sections. Note that CD5-positive T-cells were distributed diffusely

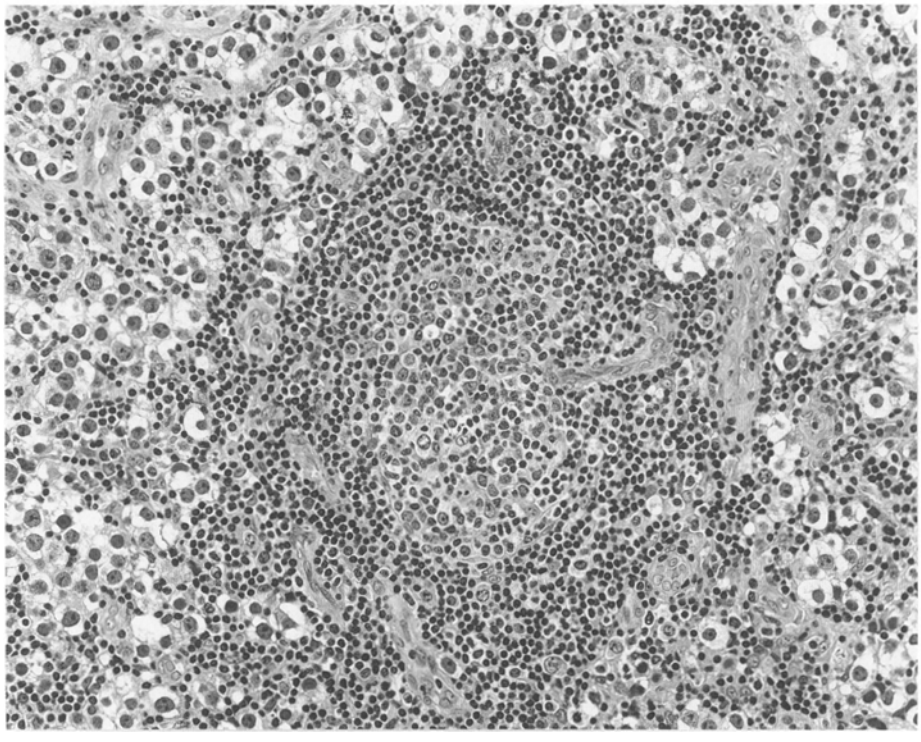

Fig. 2. Haematoxyline and eosin staining. Tumour infiltrating lymphocytes (TIL) have accumulated and formed a lymphoid follicle

at 1:40 dilution were applied and incubated similarly. Final washing was followed by colour reaction with 3,3′-diaminobenzidine tetrahydrochloride in the presence of hydrogen peroxide. The sections were counterstained by methyl-green and were observed under the microscope. Semi-quantitatively analysis was done for subsets of T-cells by counting the number of stained cells (100 cells) in high-power fields and calculating the relative ratio of CD4 to CD8.

Results

Of 20 cases with typical seminoma, TIL were identified in 18 cases. Of these 18, 12 specimens were used for immunohistological staining. Table 2 shows percentage of cells bearing lymphocyte markers as determined by monoclonal antibodies. In all 12 seminomas in which

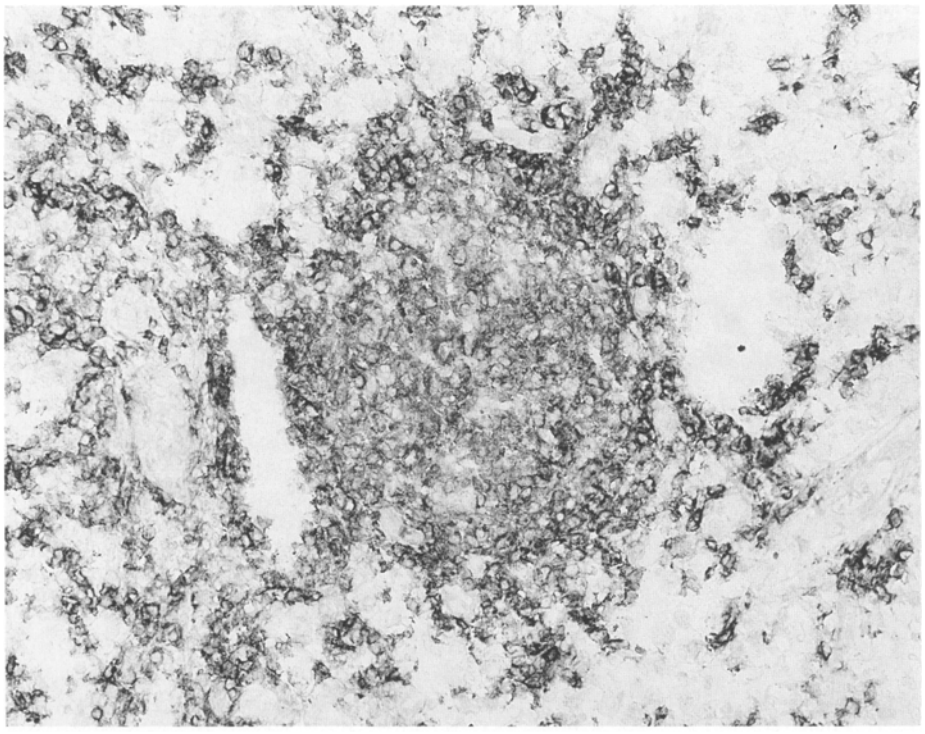

Fig. 3. L26 (CD2) staining. CD2 stained most of the cells which formed a lymphoid follicle

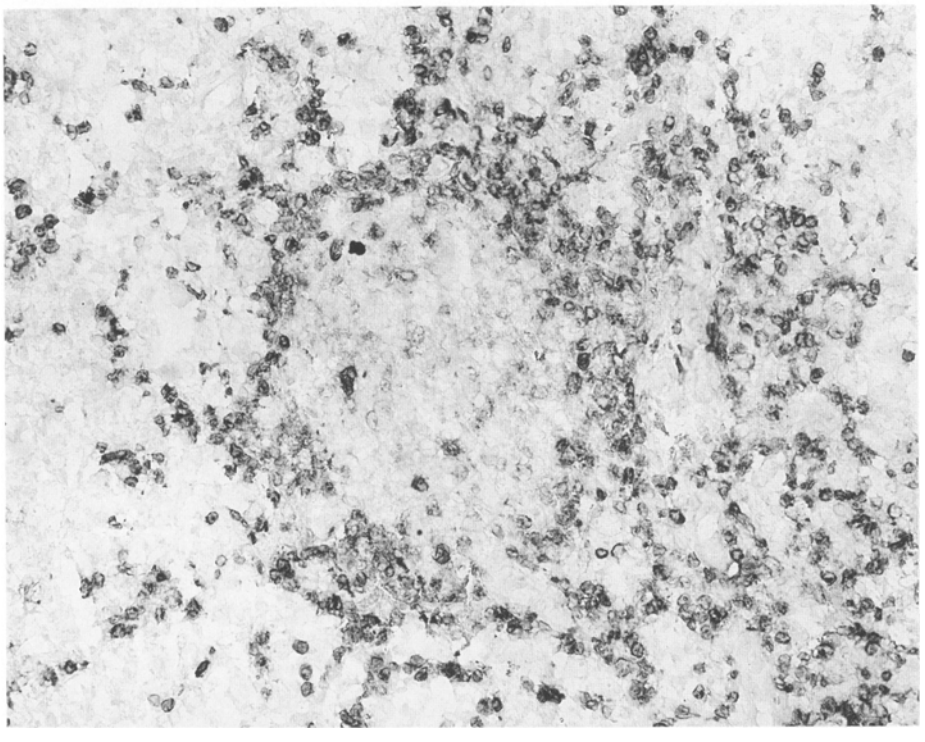

Fig. 4. L22 staining. L22 stained only the cells located in the outer part of the lymphoid follicle

TIL were shown on routine histological examinations, T-cells with pan-T-cell markers (L1) were identified (Fig. 1). No definite difference was observed when the staining pattern of Leu4 (CD3) and L1 (CD5) was compared. Generally, T-cells were distributed diffusely in the fibrovascular septa surrounding tumour cells. Functional subsets for T-cells were stained by monoclonal antibodies for CD4 and CD8. CD8+ T-cells were identified in all 12 cases, whereas CD4+ T-cells were found clearly in 5 cases and barely found in 7 cases. In addition, CD8+ T-cells were more numerous than CD4+ T-cells.

To clarify this observation, semi-quantitative analysis was performed and CD4/CD8 ratio was determined by means of previously mentioned method. As is shown

Table 2. Cases with cells bearing lymphocyte markers as determined by monoclonal antibodies

CD5 L1 pan-T	CD3 Leu4 pan-T	CD8 L2 Cytotoxic-suppressor T	CD4 L3 Helper-inducer T	CD2 L26 pan-B	L22 B-subset
12/12	12/12	12/12	5/12	8/12	12/12

Table 3. T-cell subset in tumour infiltrating lymphocytes

Patient	CD4/CD8	Patient	CD4/CD8
1	0.17	8	0.55
2	0.04	9	0.05
3	0.14	10	0.03
4	0.14	11	0.06
5	0.05	12	0.04
6	0.09		
7	0.12	Mean	0.13

in Table 3, CD4/CD8 ratios ranged from 0.03 to 0.55, with an average CD4/CD8 ratio of 0.13. These results indicated that T-cells in TIL in seminomas were predominantly CD8+ cytotoxic/suppressor cells.

B-cells with pan-B-cell marker L26 (CD2) were clearly identified in 8 cases out of 12 seminomas with TIL. Interestingly, B-cells tended to accumulate focally and formed lymphoid follicles in 6 cases (Figs. 2, 3). In particular, L22-positive resting B-cells were found to be located in the peripheral area of the lymphoid follicles and few L22+ cells were identified in the centre of follicles (Fig. 4). The expression pattern of CD2 and L26 on these lymphoid follicles in seminoma was identical to that observed in the second lymphoid follicles in the peripheral lymphoid organs (Takami et al. 1985).

Discussion

By utilizing a panel of monoclonal antibodies, characterization of TIL in seminomas was investigated. Like previous authors (Bell et al. 1987), we found T-cells, especially CD8+ T-cells, were predominant but this result was shown in a more quantitative fashion. In 10 cases, CD4/CD8 ratio was lower than 0.5, indicating that CD8+ cells were predominant. Only 2 cases showed a ratio larger than 0.5. It was assumed that CD8+ T-cells predominated. However, it must be pointed out that there were cases of non-seminomatous germ cell tumours which showed CD4+ T-cell predominance (Gemmell et al. 1988), although the clinical significance and mechanisms are unknown.

Although lymphoid follicles were found in 18% of seminomas by Thackray and Crane (1976), previous studies have reported that B-cells were barely seen in seminoma (Bell et al. 1987; Häyry and Tötterman 1978). In contrast to those reports, B-cells were identified in TIL in seminomas in this study. By utilizing a reliable pan-B-cell marker, L26, we could clearly identify B-cells. In addition, formation of lymphoid follicles was observed, not only by histological examination but also by immunohistochemical analysis. In particular, phenotypic alternation was found to occur in such lymphoid follicles as revealed by the distribution pattern of L22. In the secondary follicles of the lymphoid organs, L22 labels the B-cells located in the mantle zone B-cells and the germinal centre B-cells were mostly reactive with this antibody, thus indicating that L22 is a useful marker for resting B-cells (Takami et al. 1985). It is noteworthy that we observed lymphoid follicles in seminoma with similar expression pattern of L22 and L26 to those of normal lymphoid follicles seen in tonsillas and intestine. These findings suggest that the lymphoid follicles observed in seminoma were structurally and immunophenotypically mature.

Identification of lymphoid follicles in seminoma and the expression pattern of L22 and L26 in such a structure clearly indicated that B-cells infiltrating into seminomas differentiate, both morphologically and functionally.

Lymphoid follicle formation in TIL has been observed in other types of tumours such as medullary carcinoma of the breast (Shimokawara et al. 1982) and gastric cancers (Shimamura et al. 1977). In such cases, the prognosis of the disease has been reported to be better than cancers with no follicle formation. In this regard, it is important to know whether lymphoid follicle formation in seminomas as well as other types of stromal reaction (macrophages; Schütte et al. 1988) relates to the biological behaviour of seminoma.

The mechanism by which lymphocytes migrate into the site where tumour cells exist is not well described. Recent experimental studies have demonstrated that homing of lymphocytes to specified sites involves an interaction between lymphocytes and endothelial cells and that functional maturation of lymphocytes is regulated by a variety of lymphokines. For example, the presence of a factor produced by helper T-cells in situ which induces the maturation of cytotoxic T-cells has been described (Uede et al. 1985). Therefore, the presence of phenotypically different subsets of T-cells and B-cells implies the existence of complicated mechanism of cell-to-cell interaction induced by tumour cells. Whether TIL in seminoma represents an anti-tumour response is not known but further functional analysis of TIL is necessary for a better understanding of the biology of seminoma and for the development of potentially new therapeutic methods.

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