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Cellular characteristics of neoplastic angioendotheliosis

An immunohistological Marker study of 6 cases

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Summary. Neoplastic angioendotheliosis (NAE) is a rare, mostly fatal disease characterized by proliferation of large blastoid cells in small vessels of various organs. The origin of neoplastic cells remain undetermined. In this study, cell markers were studied immunohistologically on paraffin sections of six cases of NAE, by applying avidin-biotin-peroxidase (ABC) method and five antibodies which can demonstrate marker antigens on formalin fixed and paraffin embedded specimens. It was shown that the neoplastic cells were heavily stained with an anti-B lymphocyte monoclonal antibody LN-1 (6/6), moderately stained with another anti-B lymphocyte antibody LN-2 (5/6) and heavily stained with a monoclonal antibody which reacts with all levels of leukocytes (Dako-LC) (6/6). The cells did not show positive reaction with an anti-myelomonocytic antibody anti-Leu M1. The reaction against anti-Factor VIII, which can depict endothelial cells, was mostly negative, and if positive, was faint and undefinite, leading to an assumption that the reaction was against antigens in serum and not against neoplastic cells. These results suggest that the neoplastic cells of NAE are in the B lymphocyte lineage.

Key words: Lymphoma – B lymphocyte – Haemangioendothelioma – Endothelium

Introduction

Neoplastic angioendotheliosis (NAE) is a rare, mostly fatal disease characterized by obstruction of small blood vessels of various organs by intralumi-

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nal infiltration of apparently neoplastic blastoid cells (Pfleger and Tappeiner 1959; Scott et al. 1975; Wick et al. 1981). The origin of these cells remains unclear: Many authors suggested that they are of endothelial origin (Scott et al. 1975; Petito et al. 1978; Arnn et al. 1980; Wick et al. 1981, 1982; Filling et al. 1983), while others regarded them as lymphoid malignancies (Ansell et al. 1982; Scott et al. 1975), or even carcinoma from an occult site (Dolman et al. 1979).

The authors recently reported a case of NAE whose neoplastic cells were of the B lymphocyte lineage (Mori et al. 1985). It was revealed in this study that the neoplastic cells in small vessels bear monoclonal surface immunoglobulin μ, λ, together with other B cell markers such as B1, Leu14, Dako-panB and (OK)B2.

Further studies were awaited to conclude if the NAEs are the B cell malignancies in general. Such studies, however, could not be performed because most of NAE cases had been diagnosed at autopsy, i.e. after specimens had been fixed with formalin and embedded in paraffin, while most of the anti-B lymphocyte antibodies could not react with antigens denatured by such treatments.

The LN-1 and LN-2 are newly developed monoclonal antibodies which demonstrate B lymphocytes on B5 formalin-fixed and paraffin embedded sections (Epstein et al. 1984; Marder et al. 1985). In the present study, these antibodies were introduced and five additional cases of NAE were examined, in order to determine whether such cases also belong to B cell malignancy.

Materials and methods

Two cases (case # 2 and # 3 on Table 1) were selected from our autopsy records. Three additional cases (# 4, # 5, # 6), which had been autopsied at Department of Pathology, Tokyo Medical and Dental College, were also examined by the courtesy of Dr. M. Kitagawa and Professor T. Kasuga. Pathological reexamination disclosed all of these cases to be typical NAEs: Small vessels of various organs were filled with apparently neoplastic blastoid cells (Figs. 1, 2).

The following cases were used as controls: Skin biopsy of a case of NAE whose neoplastic cells had been shown to be in B lymphocyte lineage (case # 1); five B cell lymphomas; three T cell lymphomas; one null cell lymphoma. Two apparently normal peri-gastric lymph nodes were also used as controls.

To demonstrates markers on these neoplastic cells, four monoclonal antibodies (LN-1, LN-2, Dako-LC, anti-Leu M1) and one hetero-antibody (rabbit anti-Factor VIII, Dako) were used: LN-1 reacts with B lymphocytes, and particularly of germinal center B lymphocytes level (Epstein et al. 1984); LN-2 demonstrates chiefly B lymphocytes of mantle zone cell level (Epstein et al. 1984); Dako-LC (leukocyte-common) reacts basically with all leukocytes including lymphocytes, granulocytes and monocytes (Dalchau et al. 1980); anti-Leu M1 can show myelo-monocytic cells (Hanjan and Kearney 1982); Factor VIII is shared by megakaryocytes, platelets and endothelial cells (McComb et al. 1982). All these antibodies can demonstrate antigen-bearing cells on formalin-fixed and paraffin-embedded tissues.

Immunostaining was performed following the method of Hsu et al. (1982). Antibodies listed above were used as the first reagent, and the ABC staining kits (Vector, USA) for mouse and rabbit immunoglobulins were introduced as the second and third reagents. Briefly, four μ sections were cut from the paraffin blocks of these cases. They were de-paraffinated with xylene. Tissue peroxidase on these sections was blocked by rinsing slides with 3% H_2O_2

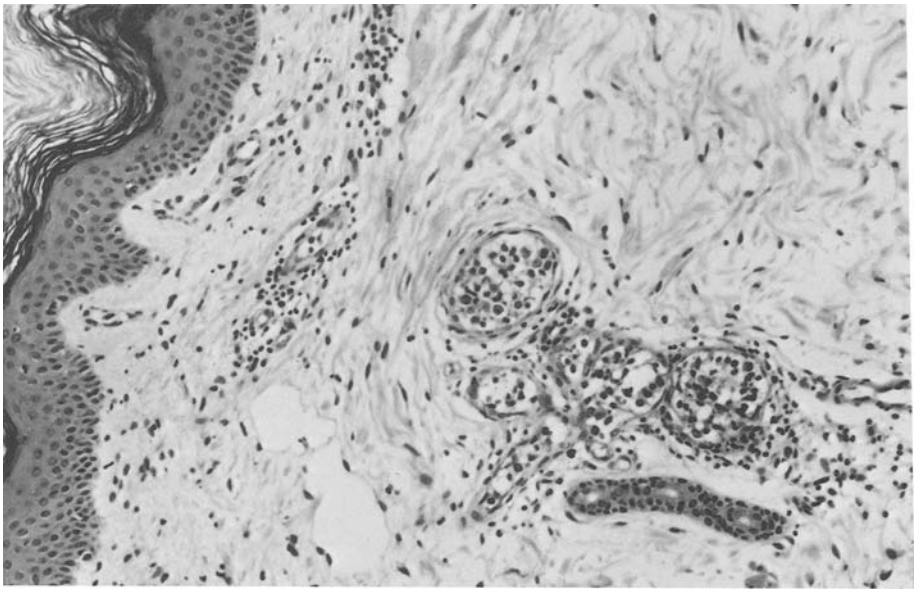

Fig. 1. Skin lesion of neoplastic angioendotheliosis (NAE). Small vessels of dermis are filled with abnormal cells. (Case # 1, × 60)

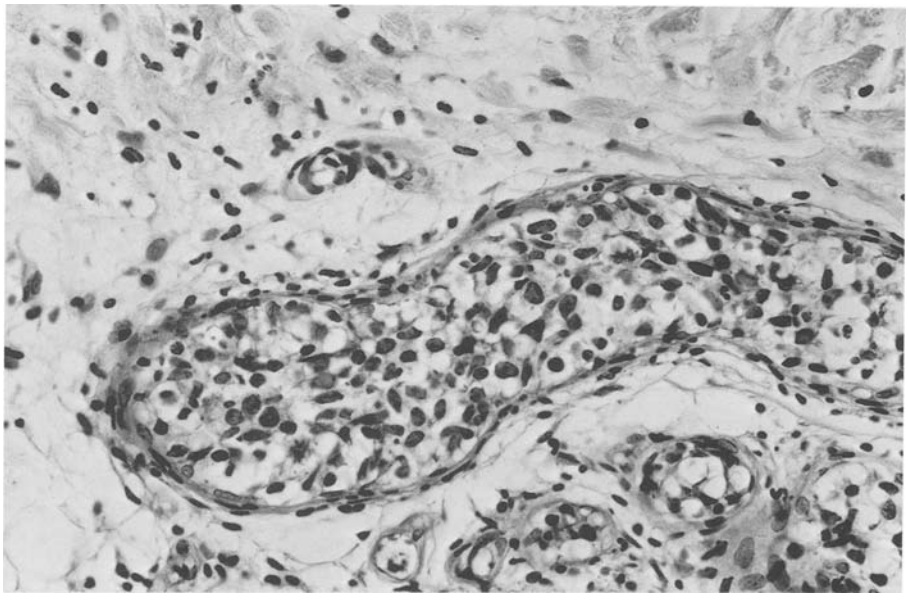

Fig. 2. Higher magnification of Fig. 1. The abnormal cells are composed of apparently neoplastic large blastoid cells. (× 300)

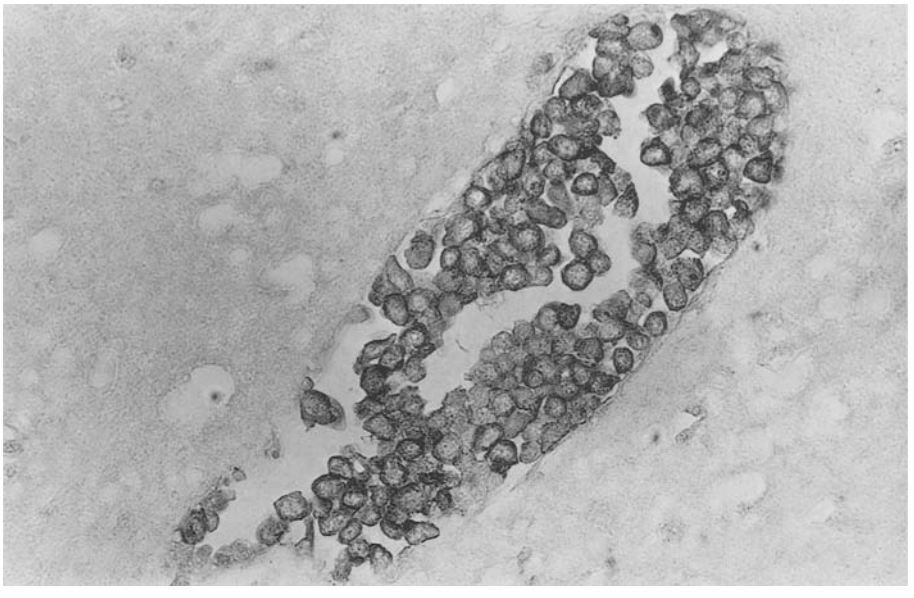

Fig. 3. Reaction of neoplastic cells of NAE to LN-1. (Case # 6, × 600)

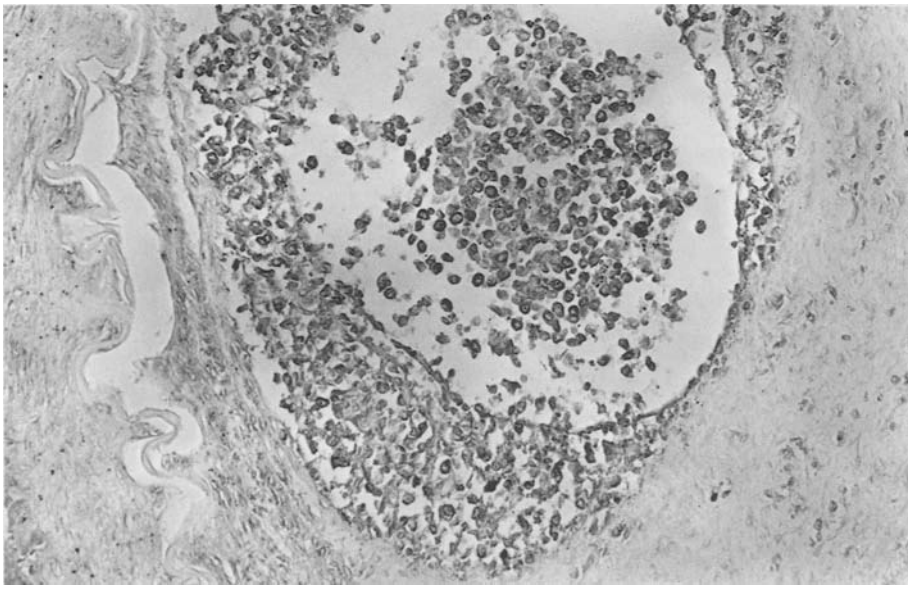

Fig. 4. Reaction to LN-2. Some of the neoplastic cells of this case are infiltrating subendothelially. (Case #3, × 150)

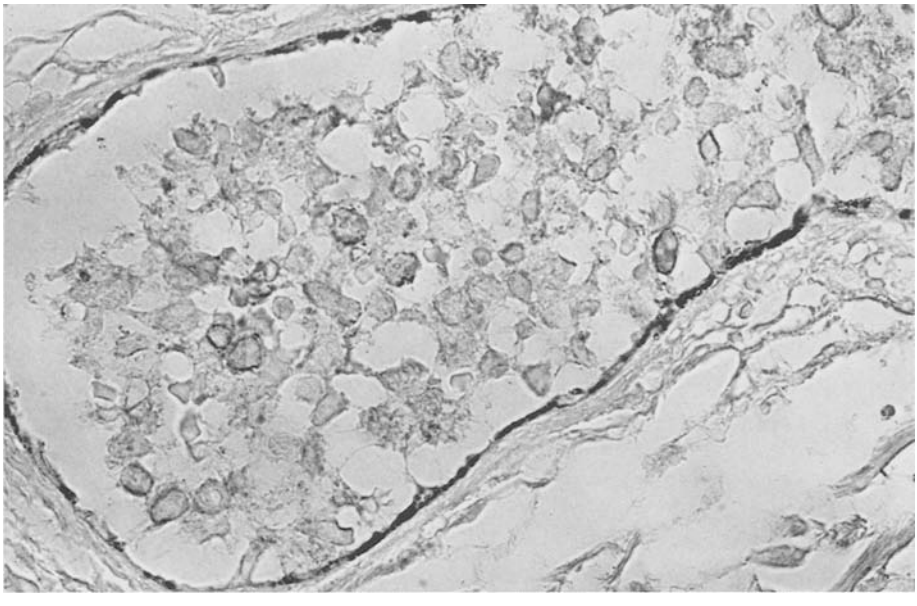

Fig. 5. Reaction to anti-Factor VIII. Endothelial cells are intensely stained, while a few of the cells in vascular lumina are faintly stained. (Case # 1, ×600)

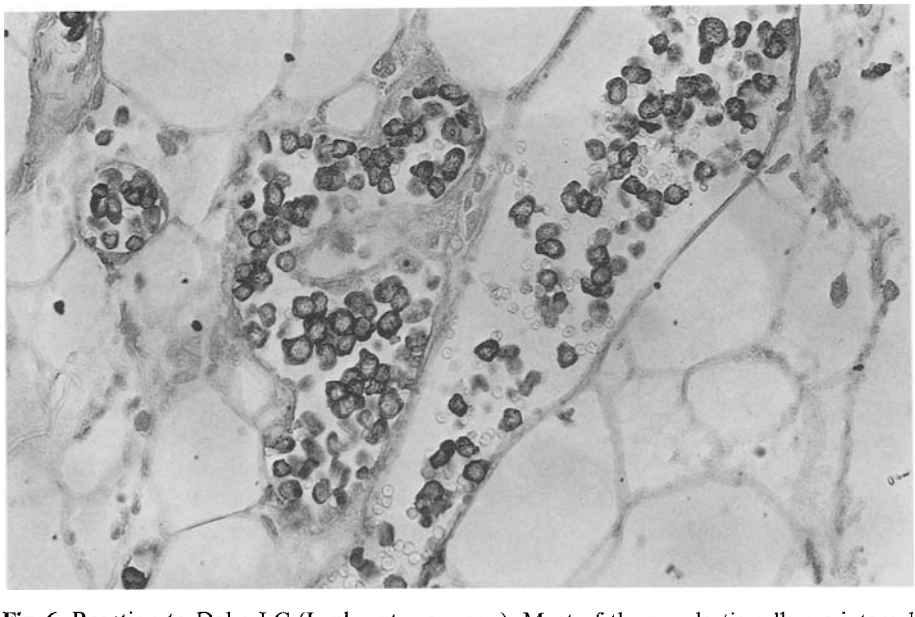

Fig. 6. Reaction to Dako-LC (Leukocyte-common). Most of the neoplastic cells are intensely stained. (Case # 5, periadrenal soft tissue, ×200)

Table 1. Reaction of neoplastic cells to various antibodies

A. Neoplastic angioendotheliosis (NAE)

Case	Examined organ	LN-1	LN-2	Factor VIII	LC	M1
# 1 64F	skin	3,+ +	3,+ +	1,+	3,+	−
# 2 63M	testis	3,+ +	1,+	1,+	2,+	−
# 3 59M	brain	3,+ +	3,+ +	2,+	3,+ +	−
# 4 67F	lymph node	3,+ +	3,+ +	−	3,+ +	−
# 5 77M	adrenal	3,+ + +	3,+ +	1,+	3,+ + +	−
# 6 50M	brain	3,+ + +	3,+	1,+	3,+ +	−

B. Control cases

Case	Marker	LN-1	LN-2	Factor VIII	LC	M1
# C1 ML. Foll	B (μ, k)	3,+ +	3,+ +	−	3,+ +	−
# C2 ML. Foll	B (μ, k)	3,+ +	3,+ +	−	3,+ +	−
# C3 ML. Burk	B (μ, k)	3,+ +	n.d.	−	3,+ +	−
# C4 ML. DL	B (μ, λ)	n.d.	3,+ +	1,+	2,+	−
# C5 ML. DS.	B (μ, k)	3,+ +	3,+ + +	−	2,+ +	−
# C6 ML. DS.	T (Leu1, 3a, 4)	−	1,+	−	2,+	−
# C7 ML. D. Pleo	T (Leu1, 3a, 4)	−	−	−	−	−
# C8 ML. D. Pleo	T (Leu1, 3a, 4)	−	−	−	2,+	−
# C9 ML. D. Lbl	Null (Ia, OKT9, 10)	−	3,+ + +	1,+	1,+	−

Numbers indicate the ratio of positive cells in total nucleated cells. 3: most of the nucleated cells are positive, 2: less than one half are positive, 1: a few cells are positive, −: no positive cells. Crosses indicate the intensity of staining on positive cells. + + +: heavily stained, + +: moderately stained, +: faintly stained. ML: malignant lymphoma, Foll: follicular, D: diffuse, S: small cell, L: large cell, Burk: Burkitt, Pleo: pleomorphic, Lbl: lymphoblastic.

in ethanol. Slides were then incubated with the first, second and third reagents succeedingly, with wash by phosphate buffered saline after each steps. Diamino-benzidine was used as chromogen. Methyl green was used for counterstaining.

Results

Neoplastic cells of all six cases of NAE showed positive reactions with LN-1. The reaction was clear enough, although there seem to exist some differences in intensity among the neoplastic cells (Fig. 3). In control slides, LN-1 demonstrated germinal center cells and B lymphoma cells clearly, while it did not react with most of the mantle zone B-cells, small lymphocytes in T-cell areas of normal lymph nodes, T lymphomas or null cell lymphomas.

LN-2 showed positive reactions with five out of six cases of NAE (Fig. 4). As background stain was much more intense on LN-2, the staining was not so clear compared with LN-1. In control slides, LN-2 demonstrated mantle zone cells and T-zone histiocytes most intensly. It also reacted with germinal center B cells and B lymphoma cells definitely, while it did not react with normal T cells or T-lymphomas.

Factor VIII was demonstrated heavily on endothelial cells of all the slides examined. This factor was not demonstrated on most of the neoplastic cells of NAE. However, a part of the cells in blood vessels, shown in Fig. 5, were slightly stained by this antibody. On such slides, the number of stained cells were small and the reaction was very weak. Such a reaction was observed in a few of control B- and null cell lymphomas as well.

All the neoplastic cells of NAE and B cell lymphoma were shown to be intensely stained by Dako-LC (anti-leukocyte common antibody) (Fig. 6). A part of T cell lymphomas and a case of null cell lymphoma were faintly stained by this antibody. None of the neoplastic cells examined in this study were stained by anti-Leu M1, while granulocytes and macrophages were clearly shown. The results are summarized on Table 1.

Discussion

LN-1 is known to demonstrate B lymphocytes, and particularly germinal center B lymphocytes, while it also cross-reacts with glandular epithelia of various organs. LN-2 reacts with B lymphocytes, null lymphocytes and some histiocytes but not with epithelia. Both of these antibodies stain B cell lymphomas beautifully while they do not stain T cell lymphomas (Marder et al. 1985). None react with endothelial cells (Epstein et al. 1984). In the present study, neoplastic cells of NAEs were shown to react heavily with LN-1 and moderately with LN-2. It should be noted that neoplastic cells of a case (case # 1), which had been shown to be a B cell neoplasm by the presence of surface monoclonal immunoglobulins (Mori et al. 1985), were stained with LN-1 and LN-2 in the same pattern as the other NAEs. These results strongly suggest that the neoplastic cells of the NAEs are in the B lymphocyte lineage. It might be worthwhile to comment that these results are not conclusive; demonstration of the monoclonality of surface immunoglobulins (Igs) will be needed for final justification in calling them a B cell malignancy. This, as mentioned above, could not be performed because such Igs had lost antigenicity during fixation and embedding. In this context, our previous report (Mori et al. 1985) was the first and the only one case so far in which the monoclonality of Igs was demonstrated in situ, while Ansell et al. (1982) demonstrated surface IgM on circulating atypical cells in their case of NAE. In any case, it might be said that the present results are enough to assume NAEs to be B cell malignancies.

More discussions might be needed before accepting this assumption. Many previous reports have suggested that NAEs are of endothelial origin (Scott et al. 1975; Petito et al. 1978; Arnn et al. 1980; Wick et al. 1981, 1982; Filling et al. 1983). In a large part of these reports, such an assumption was based upon pure morphology. However there are several reports which tried to confirm it by using an endothelial marker Factor VIII and in these reports, the results of imunostaining are not unanimous: some reports stressed the presence of Factor VIII on neoplastic cells (Arnn et al. 1983; Fulling and Gersell 1983); while others failed to demonstrate it (Wick et al. 1981, 1982; Beal and Fischer 1982). In the present study, Factor VIII was

demonstrated heavily on vascular endothelium and weekly on a few cells in vascular lumina. The number of positive cells in vascular lumina was very small and the staining was faint. Furthermore, there is a case whose neoplastic cells were definitely Factor VIII negative on frozen sections but were shown to be partially positive on formalin-fixed sections (case # 1). These results will be sufficient to assume that NAEs are basically negative for Factor VIII. In some of NAEs, how come these positive cells? As Factor VIII is rich in plasma, it is quite possible that serum Factor VIII was fixed on surface of cells in the vascular lumina when specimen was placed in fixative, while it was washed away on frozen sections. Thus, it is more likely that such a reaction simply shows the factor in serum. In summing up this part of discussion, as the Factor VIII is basically negative, while antibodies which do not react with endothelial cells, such as LN-1, LN-2 and Dako-LC, react with those cells, it is reasonable to assume that the neoplastic cells are not of vascular origin.

Assuming that the neoplastic cells are B lymphocytes, they will be placed in some special level of differentiation, as the immunohistological and morphological features of the six cases were closely similar. However, the present study could not show at what level of differentiation they are, nor could it clarify the mechanism which permits these neoplastic cells to remain in vascular lumina.

It might be worthwhile to add that this assumption gives help in producing rational therapy for NAEs. There are a few reports in which antileukaemics were successfully used on NAEs (Scott et al. 1975; Keahey et al. 1982; Shiozaki et al. 1983). If NAEs are a B cell malignancy, this therapy is quite reasonable, and will be recommended in further cases.

Finally it may be suggested that the term neoplastic angioendothelio(mato)sis should be discarded. These cases should now be described under a term which implies the nature of neoplastic cells more precisely, such as malignant lymphoma mainly involving vascular lumina, or another better term.

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