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A Novel Monoclonal Antibody (OPD4) Recognizing a Helper/Inducer T Cell Subset

Its Application to Paraffin-Embedded Tissues

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A novel monoclonal antibody (MAb), OPD4, reactive with a helper/inducer (H/I) subset of T cells in formalin-fixed, paraffin-embedded tissue sections, has been identified through immunization with an activated H/I T cell line, namely DL40. The antibody is an IgG1 antibody and it recognizes an antigen with a molecular weight of 200 kd, corresponding to that of leukocyte common antigen. OPD4$^+$/CD4$^+$ T cells provided better help for pokeweed mitogen-stimulated polyclonal IgG production than OPD4$^-$/CD4$^+$ T cells. OPD4 recognized the H/I T cell subset even in paraffin-embedded tissue sections, but did not recognize nonhematopoietic cells, suppressor/cytotoxic T cells, B cells, monocytes in the peripheral blood, or other normal hematopoietic cells as examined by the flow cytometric and immunoperoxidase methods. Besides the lymphoid cells, OPD4 reacted with a number of histiocytes (epithelioid cells) in tissues from sarcoidosis and tuberculosis. For the neoplastic lesions, OPD4 reacted with approximately half of the cases of T cell lymphomas. Consequently, OPD4 may be useful for the diagnosis and study of malignant lymphomas and other related lesions. (Am J Pathol 1989, 134:1339–1346)

A large number of monoclonal antibodies (MAbs) have been developed against hematolymphoid cells to characterize the phenotype in connection with cell differentiation. These MAbs are valuable for the immunophenotypic diagnosis of hematolymphoid malignancies, but most MAbs are available only for frozen sections or suspended live cells. Cryostat sections have the disadvantage of poor preservation of cell morphology. Dispersed cell preparations have the risk of not dealing with neoplastic cells when there exist a large number of intermingled non-neoplastic cells.

Recently MAbs have been studied that react with formalin-fixed, paraffin-embedded materials. These include MAb to leukocyte common antigen (LCA, CD45) for a lymphoid origin,[1] MT 1,[2] and UCHL 1[3] for T cells, and MB1[2] and LN series[4] for B cells. These MAbs are useful and have been widely used. To date, however, there have been no reliable antibodies that recognize certain T cell subsets.

This report describes a new MAb, designated OPD4, that recognizes a subset of CD4$^+$ peripheral T cells with helper function even in formalin-fixed, paraffin-embedded tissues.

Materials and Methods

Cells and Tissues

Mononuclear cells and granulocytes were separated from peripheral blood. T cells forming rosettes with sheep erythrocytes were isolated and B cells and monocytes were separated by culture in a glass dish. Nonhematolymphoid cell lines examined were HeLa (cervical squamous cell carcinoma), HGC-25 (pancreatic adenocarcinoma), HLC-1 (pulmonary adenocarcinoma), MCT-7 (mammary adenocarcinoma), PL-14 (melanoma), KG-1-C (glioma), and YT-nu (neuroblastoma).

Normal, neoplastic, and other pathologic human tissues were obtained by surgical excision. Tissues for the frozen section studies were frozen in embedding compounds. Tissues for paraffin embedding were fixed in 10% buffered formalin.

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Immunization

A lymphoma cell line, DL40,[5] was used as an immunogen. Balb/C mice were immunized twice with 10^7 DL40 cells emulsified in complete Freund's adjuvand and were boosted with 10^7 DL40 cells without adjuvand 3 days before they were killed.

Cell Fusion and Cloning

Spleen cells of immunized mice were fused with P3X63 Ag 8.653 mouse myeloma cells according to a standard protocol.[6] Culture supernatants were screened by immunoperoxidase staining on paraffin-embedded nonspecific hyperplastic lymph nodes and other organs and acetone-fixed cell lines. Positive hybridoma colonies were serially cloned three times. Ascitic fluid, containing a high titer monoclonal antibody, was obtained and purified IgG was obtained with protein-A sepharose chromatography (Nippon Bio-Rad Lab. Co. Ltd. Tokyo, Japan).

Serologic Determination of MAb Isotype

Heavy chain subclass of the OPD4 was determined by the Ouchterlony method using antisera specific for each mouse heavy chain class (Zymed Lab. Inc., San Francisco, CA).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

NP40-solubilized DL40 cells were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE)[7] and transferred to a GVHP membrane (Pharmacia, Uppsala, Sweden).[8] After blocking, the membrane was incubated with OPD4 (18 μg/ml) for 2 hours and then with horseradish peroxidase-conjugated anti-mouse IgG (Tago Inc, Burlingame, CA). Polyclonal IgG from Balb/C mice and a cell line, BALL, were used as antibody and antigen controls, respectively. The peroxidase was detected by 1.26 mM 3.3′-diaminobenzydine tetrahydrochloride and 0.005% H_2O_2.

Immunophenotyping Studies Using Flow Cytometry

Approximately 10^6 mononuclear cells from healthy donors were incubated with 100 μl of monoclonal antibody for 30 minutes at 4 C. Cells were washed and incubated with fluorescein isothiocyanate (FITC)-labeled $F(ab')_2$ fragment of goat anti-mouse IgG diluted 1:20 (Tago Inc). After further washing, cells were allowed to react with 100 μl of phycoerythrin (PE)-conjugated anti-T cell and B cell monoclonal antibodies for 30 minutes at 4 C. The PE-conjugated monoclonal antibodies included Leu-1(CD5), 2a(CD8), 3a(CD4), 4(CD3), and 16(CD20) (Becton-Dickinson, Mountain View, CA). The samples were analyzed by Epics 750 (Coulter Electrics, Inc., Hialeah, FL) with simultaneous excitation using 488 nm argon laser (300 mW). The analysis was gated by using forward and 90° light scatter to exclude dead cells and to estimate comparative reactivity between the lymphocytes and the monocytes.

Functional Analysis

To determine the effect of fractionated T cells on antibody production, 10^4 to 10^5 $CD4^+/OPD4^+$ or $CD4^+/OPD4^-$ T cells, obtained separately by aseptic sorting, were added to 10^5 CD 20^+ cells in round-bottomed microtiter trays. Culture was done in 200 μl of RPMI 1640 supplemented with 10% FCS and 0.05% PWM. After incubation for 5 days, supernatants were harvested and assayed for the presence of IgG by solid phase enzyme-linked immunosorbent assay (ELISA).[9] The IgG concentration in the supernatant was calculated from the log/linear regression obtained from serial dilutions of a standard of known IgG concentration.

Immunoperoxidase Staining

Cell smears were fixed in cold acetone or 10% buffered formalin. Frozen sections were made with a cryostat and subjected to cold acetone fixation. Formalin-fixed, paraffin-embedded specimens were cut at 3 to 4 μ. After blocking endogenous peroxidase activity with 0.3% H_2O_2-methanol, all sections were stained with OPD4 (1.8 mg/ml of purified IgG was used at a dilution of 1:200) by using the avidin-biotin immunoperoxidase staining procedure (Vector Laboratories, Inc., Burlingame, CA) as described by Hsu and coworkers.[10]

Results

Development of MAb OPD4

Balb/C mice were immunized with DL40 cells established from diffuse large cell type lymphoma. Immunophenotyping and other cytologic studies of DL40 cells revealed that they were E^+, $CD4^+$, $CD5^-$, $CD8^-$, $CD10^-$, $CD14^-$, $CD19^-$, $CD25^+$, Leu-$5b^+$, Leu-7^-, HLA-DR^+, surface immunoglobulin $(Ig)^-$, cytoplasmic Ig^-, terminal deoxynucleotidyl transferase$^-$, Epstein-Barr (EB) virus-associated nuclear antigen$^-$, and Adult T cell Leukemia (ATL) associated antigen$^-$.

Isotypic analysis revealed that OPD4 was of an IgG1 heavy chain subclass. As shown in Figure 1, Western blotting analysis of the cell lysates revealed that molecular weight of the antigen recognized by OPD4 was 200 kd.

Cytofluorometric Analysis of OPD4 Reactivity with Normal Peripheral Blood Cells

Dual parameter analyses revealed that approximately 31% of peripheral $CD3^+$ lymphocytes and 52% of $CD4^+$ lymphocytes expressed the OPD4 antigen (Figure 2). On the contrary, only 1.2% of $CD8^+$ lymphocytes and 0.8% of $CD20^+$ lymphocytes were $OPD4^+$. OPD4 did not react with monocytes as shown by gating analysis.

Helper Function of $OPD4^+$ T Cells

As shown in Table 1, $OPD4^+/CD4^+$ T cells ($D4^+$) provided better T cell help than $OPD4^-/CD4^+$ ($D4^-$) for PWM-driven polyclonal IgG production; the amount of IgG (ng/ml) induced in the presence of $D4^+$ or $D4^-$ was 1329/855 (10^5 cells added) and 1206/818 (10^4 cells added).

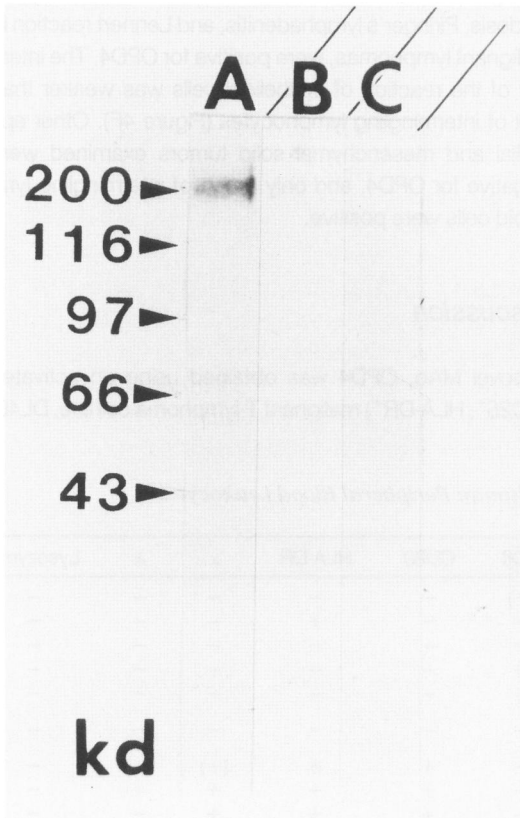

Figure 1. *Western blot analysis of OPD4. Lane A: DL 40 extracts reacted with OPD4. Lane B: BALL-1 extracts reacted with OPD4. Lane C: DL 40 extracts reacted with mouse IgG as a control. A band corresponding to a molecular weight of 200 kd can be noted in lane A.*

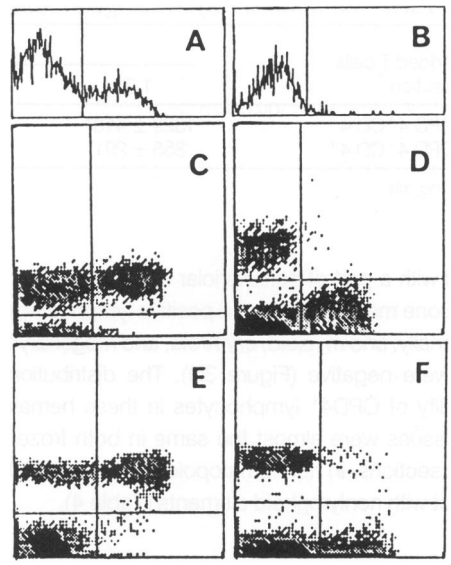

Figure 2. *Flow cytometric analysis of peripheral blood lymphocytes (PBL) from a healthy donor, labeled with OPD4-FITC and an other lymphocyte marker-PE.* **A, B:** *One parameter analysis of PBL labeled with OPD4-FITC. The x-axis represents staining intensity and the y-axis represents cell number. Gating analysis was performed against monocytes in panel B.* **C to F:** *Dual color immunofluorescence analysis of PBL with OPD4-FITC (green fluorescence) on the x-axis and Leu-4 (CD3) (C), Leu-16 (CD 20) (D), Leu-3a (CD4) (E), or Leu-2a (CD8) (F) -PE (red fluorescence) on the y-axis plotted in logarithmic units*

OPD4 Reactivity with Normal Blood Cells, Cell Lines, and Normal Tissues

The reactivity of OPD4 on normal human peripheral blood leukocytes and established human hematolymphoid cell lines is shown in Table 2. OPD4 antigen was present in a part of normal peripheral T cells and the T cell lines that expressed a helper/inducer (H/I) phenotype, but was not detected in normal and cultured neoplastic B cells, monocytes, and granulocytes. There was no difference in reactivity between the formalin-fixed and the acetone-fixed materials. None of the solid tumor cell lines examined was reactive with OPD4.

Table 3 summarizes the reactivity of OPD4 with normal hematolymphoid tissues. OPD4 produced strong membrane staining of lymphocytes in the T cell areas of lymph nodes and tonsils (Figure 3A). There also were positive lymphocytes within the germinal centers and a few in the mantle zones (Figure 3B).

The distribution of $OPD4^+$ cells in the tonsils was the same as that of $CD4^+$ lymphocytes. In thymuses, some but not all lymphocytes both in the cortex and medulla were positive for OPD4 (Figure 3C). In the spleen, OPD4

Table 1. *PMW—Stimulated Polyclonal IgG Production Augmented by Fractionated T Cells*

Added T cells fraction	No. of T cells added to 10^5 B Cells ($\times 10^5$)			
	1.0	0.4	0.1	0
OPD 4^+CD 4^+	1329 ± 418*	1206 ± 145	620 ± 314	199 ± 101
OPD 4^-CD 4^+	855 ± 291	818 ± 360	488 ± 95	181 ± 142

* IgG (ng/ml).

reacted with a part of periarteriolar and red pulp lymphocytes. Bone marrow contained positive lymphocytes only occasionally, and myeloid, erythroid, and megakaryocytic series were negative (Figure 3D). The distribution and stainability of $OPD4^+$ lymphocytes in these hematolymphoid tissues were almost the same in both frozen and paraffin sections. In nonhematopoietic organs, OPD4 did not react with nonlymphoid elements (Table 4).

OPD4 Reactivity with Non-neoplastic and Neoplastic Lesions

Immunoperoxidase staining reactivity of OPD4 on malignant lymphoma/leukemia specimens is shown in Table 5. Fifty-three percent of malignant lymphomas of T cell phenotype were positive for OPD4. In mycosis fungoides and adult T cell lymphoma/leukemia (ATL), which are generally thought to belong to a H/I T cell subset, three (50%) and seven cases (88%), respectively, were positive for OPD4. The $OPD4^+$ lymphoma cells exhibited the same membrane-bound staining pattern as the normal $OPD4^+$ lymphocytes (Figure 4). In positive cases, the majority of lymphoma cells were positive for OPD4, and the intensity of reactivity was not related to the nuclear shape and size of tumor cells. $OPD4^-$ T cell lymphoma included a case of diffuse medium-sized $CD8^+$ T cell lymphomas. In B cell lymphomas two exceptional cases (2%), one of which was a follicular lymphoma and the other a diffuse large cell lymphoma, were positive for OPD4. The others were unreactive although $OPD4^+$ non-neoplastic mature lymphocytes were intermingled. In follicular lymphomas, the majority of interfollicular lymphocytes were $OPD4^+$; the neoplastic follicle with a poorly developed mantle zone was surrounded by a dense band of $OPD4^+$ cells (Figure 4D). On the contrary, diffuse lymphomas showed no zonal distribution of $OPD4^+$ lymphocytes. In Hodgkin's diseases, Reed-Sternberg cells were negative for OPD4, but a large number of residual non-neoplastic lymphocytes were positive for OPD4 (Figure 4E). None of the cases of myelogenous leukemias was positive for OPD4. Cells belonging to a mononuclear phagocyte system were negative for OPD4 in normal tissues but in pathologic conditions, epithelioid cells including Langhans' type giant cells, which were observed in tuberculosis, sarcoidosis, Piringer's lymphadenitis, and Lennert reaction in malignant lymphomas, were positive for OPD4. The intensity of the reaction of epithelioid cells was weaker than that of intermingling lymphocytes (Figure 4F). Other epithelial and mesenchymal solid tumors examined were negative for OPD4, and only a part of intermingling lymphoid cells were positive.

Discussion

A novel MAb, OPD4 was obtained using an activated ($CD25^+$, HLA-DR^+) malignant T-lymphoma cell line, DL40.

Table 2. *Reactivity of OPD4 and Other Antibodies with Normal Human Peripheral Blood Leukocytes and Leukemia and Lymphoma Cell Lines*

Cell	Type	OPD4	CD3	CD4	CD5	CD8	CD20	HLA-DR	κ	λ	Lysozyme
Peri. T cells		(+)	+	(+)	+	(+)	−	−	−	−	−
DL 40	T ML	+	−	+	−	−	−	+	−	−	−
CEM	T ALL	+	+	+	+	−	−	−	−	−	−
MOLT 4	T ALL	(+)	+	(+)	+	−	−	−	−	−	−
TALL 1	T ALL	−	−	−	(+)	−	−	−	−	−	−
NALL 1	Non T, non B ALL	−	−	−	−	−	−	−	−	−	−
Peri. B cells		−	−	−	−	−	+	+	(+)	(+)	−
BALL 1	B ALL	−	−	−	−	−	+	+	+	−	−
JBL	Burkitt	−	−	−	−	−	+	+	+	−	−
Peri. monocytes		−	−	−	−	−	−	+	−	−	+
J 111	AMoL	−	−	−	−	−	−	−	−	−	+
Peri. granulocytes		−	−	−	−	−	−	−	−	−	+

Data expressed as: +, positive; (+), a part of cells positive; −, negative. Peri., peripheral; ML, malignant lymphoma; ALL, acute lymphocytic leukemia; AMoL, acute monocytic leukemia.

Table 3. *Reactivity of OPD4 with Normal Lymphoid and Hematopoietic Tissues*

Lymph nodes*	
T cell zone	+++
Mantle zone	a few
Germinal center	+
Tindible macrophages	–
Interdigitating reticulum cells	–
Sinus histocytes	–
Endothelium	–
Thymuses	
Cortex	a few
Medulla	a few
Spleens	+
Bone Marrow	a few
Myeloid	–
Erythroid	–
Megakaryocytes	–

The number of positive cells ranging from – to +++.
* Tonsils showed the same distribution as lymph nodes.

Table 4. *Reactivity of OPD4 with Normal Nonhematopoietic Tissues*

Organs	Reactivity	Organs	Reactivity
Heart	Negative	Adrenal	Negative
Lung	Negative	Thyroid	Negative
Stomach	Negative*	Testis	Negative
Duodenum	Negative*	Prostate	Negative
Colon	Negative*	Ovary	Negative
Pancreas	Negative	Skeletal muscle	Negative
Liver	Negative	Cerebrum	Negative
Kidney	Negative	Cerebellum	Negative

* A part of intermingling lymphocytes in lamina propria were positively stained, but parenchymal cells and other nonlymphoid components were completely negative.

It is interesting that the cell line was $CD4^+$ but lacked pan T cell markers, CD3 and CD5. This phenotypic characteristic might have been advantageous to induce the antibody to a H/I T cell subset. The dual analyses of OPD4 and other lymphoid markers by flow cytometry disclosed that $OPD4^+$ lymphocytes belonged to H/I T cells but not to suppressor/cytotoxic (S/C) T cells or B cells. Neither granulocytes nor monocytes reacted with OPD4. OPD4 did not react with all of the peripheral H/I T cells. Functional analysis with PWM-stimulated immunoglobulin synthesis revealed that $OPD4^+$ T cells showed better helper

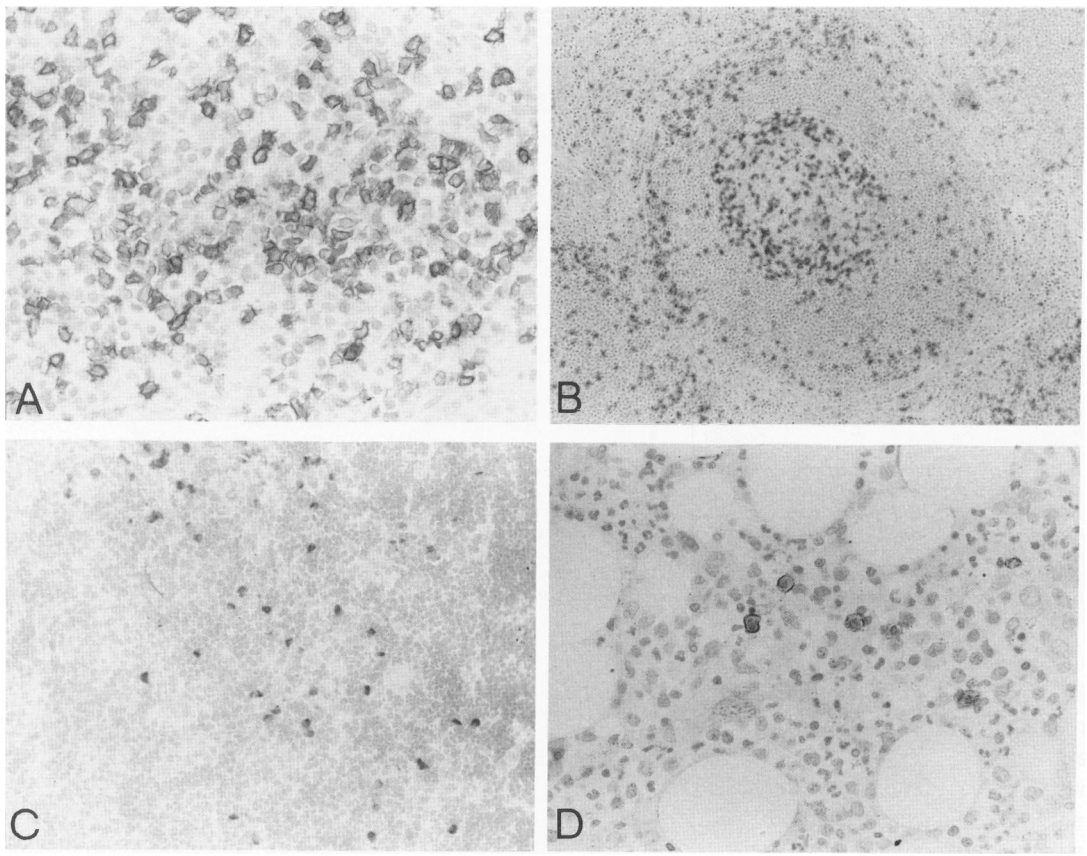

Figure 3. *Immunoperoxidase staining of OPD4 in normal hematolymphoid tissues. Panels A, B, and D are formalin-fixed, paraffin-embedded specimens. C is a frozen section.* **A:** *Considerable number of T cells in T zone area of a lymph node are positive for OPD4 (ABC method, ×280).* **B:** *T cells in the germinal center are predominantly $OPD4^+$, and a few $OPD4^+$ T cells are present in mantle zone (lymph node, ABC method, ×70).* **C:** *Some of thymic lymphocytes are positive for OPD4 (thymus, ABC method, ×140).* **D:** *Only a few $OPD4^+$ T cells are scattered in the bone marrow, whereas cells of myeloid, erythroid and megakaryocytic series are negative for OPD4 (ABC method, ×280).*

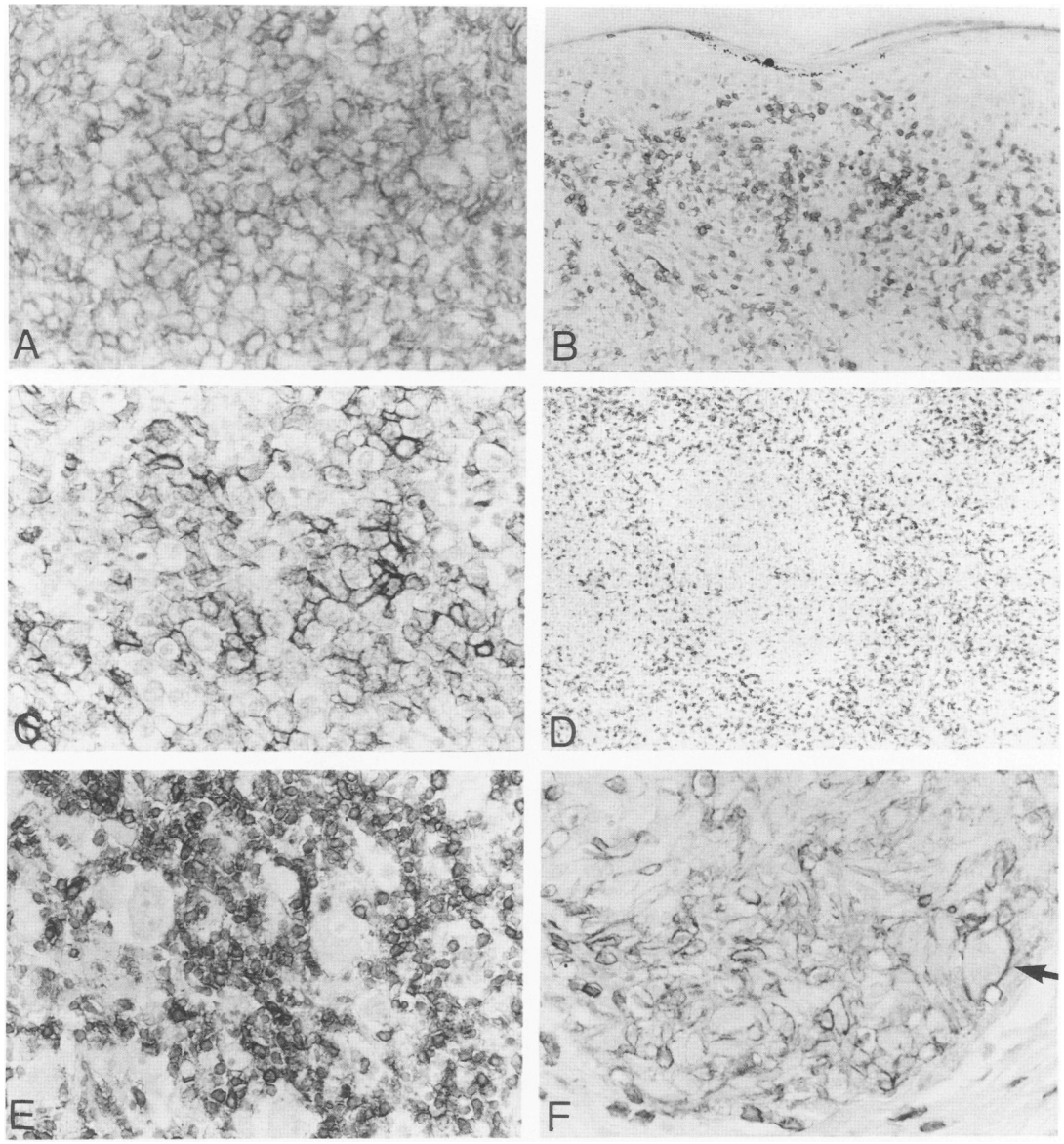

Figure 4. *Immunoperoxidase staining of OPD4 in neoplastic and other pathologic lesions.* **A:** *Diffuse large cell type (H/I T cell) lymphoma showing strong membrane-bound reaction with OPD4 (frozen section) (ABC method, ×280).* **B:** *ATL cells in various shapes and sizes infiltrating the dermis are positive for OPD4 (paraffin section, ABC method, ×140).* **C:** *OPD4 recognizes cells from clear cell lymphoma in an IBL-like T cell lymphoma (paraffin section, ABC method, ×280).* **D:** *The neoplastic follicle of a follicular lymphoma is surrounded by OPD4$^+$ T cells (paraffin section, ABC method, ×70).* **E:** *Hodgkin cells are negative for OPD4, whereas intermingling mature lymphocytes are predominantly positive for OPD4 (paraffin section, ABC method, ×280).* **F:** *Epithelioid cells and Langhans' giant cells (arrow) in a sarcoidosis are reactive with OPD4, but more weakly than intermingling T cells (paraffin section, ABC method, ×280).*

function than OPD4$^-$ T cells. Thus, OPD4$^+$ T cells belong not only phenotypically but functionally to a helper T cell subset.

These findings were confirmed by the immunoperoxidase study. OPD4 reacted with the CD4$^+$ T cell lines and normal peripheral blood T cells. The distribution of OPD4$^+$ lymphocytes in tonsils and lymph nodes was compatible with that of CD4$^+$ T cells.[12] OPD4$^+$ lymphocytes are thought to belong to peripheral H/I T cells and some thymocytes were positive for OPD4.

OPD4 recognized almost 50% of T cell lymphomas in our series. Not all cases of T cell malignant lymphomas could be subclassified immunophenotypically into H/I and S/C T cell lymphomas. As the majority of peripheral T cell lymphomas have been proven phenotypically to be of a H/I type,[13] OPD4$^+$ T cell lymphomas occupy nearly half of the H/I T cell malignancies. This ratio corresponds to a ratio of normal OPD4$^+$ T cells in H/I T cells. OPD4 reacted with approximately 90% of ATL cases. Although OPD4$^+$ T cells show helper activity, some cases of ATL cells are

functionally suppressive.[14] In our series, only 2% of B cell lymphomas were positive for OPD4; one was a follicular center cell type, and the other was an immunoblastic type. This phenomenon may be "abnormal antigen expression," as demonstrated by Picker et al.[15]

The molecular weight of the antigen defined by OPD4 was estimated to be 200 kd, which is consistent with that of leukocyte common antigen (LCA), CD45. Several antigens of the LCA group have a molecular weight around 200 kd.[16] Various types of MAbs to LCA have been reported: anti-panleukocyte antibodies (PD7/26, 2B11, F10-83-4, F8-11-13), B cell-trophic LCA antibodies (4KB5, F8-11-13), and a T cell-restricted LCA antibody (UCHL 1). Norton and Isaacson[17] suggested that commercially delivered MB1 and MT1 also were directed against LCA variants. OPD4, however, showed some different characteristics as compared with the other T cell-trophic LCA antibodies. UCHL 1^+ T cells had functional helper-inducer activity but also reacted with about 35% of $CD8^+$ T cells.[18] MT 1 and UCHL 1 are useful for the diagnosis of lymphoid malignancies, but they react not only with lymphoid cell lineage but also with other cell types. MT 1[19] recognizes macrophages, myeloid cells, and megakaryocytes, and UCHL 1[3] reacts with myeloid cells, macrophages, and various epithelial and endothelial elements. In contrast, OPD4 did not react with other hematopoietic cells such as myeloid, erythroid, and megakaryocytic series in the bone marrow, and nonhematolymphoid tissues. It is of interest that among monocytegenic macrophages, epithelioid cells including Langhans' type giant cells were positive for OPD4. The reason is now unclear.

Picker et al[15] pointed out that the immunophenotypic analysis of malignant lymphomas was sometimes the only way to make a definitive diagnosis. In some cases of malignant lymphomas, especially in T cell lymphomas, variety of histologic features, such as a large number of concomitant plasma cells, eosinophils, histiocytes, exist, which makes a definitive diagnosis difficult. OPD4 recognizes an antigen associated with H/I T cells, which is resistant to formalin fixation and paraffin embedding. Therefore, in routine histologic examination, it may be a useful tool for the diagnosis of malignant lymphomas, especially T cell malignancies.

Table 5. *Reactivity of OPD4 with Lymphoid and Hematopoietic Malignancies*

Type of malignancies	No. of cases	No. of positive cases*	Percent of positive cases
Hodgkin's disease	11	0	0
Non-Hodgkin's lymphoma (NHL)†			
B cell lymphomas	81	2	2
Follicular lymphoma	9	1	11
Diffuse lymphoma	72	1	1
T cell lymphomas	64	34	53
Diffuse medium	6	3	50
Diffuse mixed	13	5	38
Diffuse large	21	10	48
Lymphoblastic	1	1	100
IBL-like T	9	5	56
Mycosis fungoides	6	3	50
ATL	8	7	88
TB undefined	10	0	0
Myelogenous leukemia‡	5	0	0

* The reactivity with neoplastic cells was evaluated.

† NHL were classified according to the LSG (Japanese lymphoma study group) classification[11] with modification.

‡ Including three cases of chronic myelogenous leukemia and two cases of acute myelogenous leukemia.

Table 6. *Non-neoplastic Lymphoid Lesions Examined with OPD4*

Diagnosis of lesions	No. of cases
Sarcoidosis	4*
Tuberculosis	4*
Piringer's lymphadenitis	1*
Castleman's lymphoma	1
Infectious mononucleosis	1
Dermatopathic lymphadenopathy	2*
Abscess forming reticular lymphadenitis	2*
Drug-induced lymphadenitis	1
Gaucher disease	2
Necrotizing lymphadenitis	6
Immunoblastic lymphadenopathy	3
TOTAL	27

* Epitheloid cells, including Langhans' giant cells, were positive for OPD4.

References

1. Warnke RA, Gatter KC, Falini B, Hildreth P, Woolston R-E, Pulford K, Cordell JL, Cohen B, De Wolf-Reeters C, Masson DY: Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. N Engl J Med 1983, 309:1275–1281
2. Poppema S, Hollema H, Visser L, Vos H: Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte subsets in paraffin-embedded tissue sections. Am J Pathol 1987, 127:418–429
3. Norton AJ, Ramsay AD, Smith SH, Beverley PCL, Isaacson PG: Monoclonal antibody (UCHL 1) that recognizes normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol 1986, 39:399–405
4. Epstein AL, Marder RJ, Winter JN, Fox RI: Two new monoclonal antibodies (LN-1, LN-2) reactive in B5 formalin-fixed, paraffin-embedded tissues with follicular center and mantle zone human B lymphocytes and derived tumors. J Immunol 1984, 133:1028–1036
5. Kubonishi I, Hirose S, Iwahara Y, Yoshimoto S, Yamato K, Sonobe H, Taguchi H, Ohtsuki Y, Miyoshi I: Establishment of a human cell line with activated T-cell phenotype (Leu $3a^+$, $OKla1^+$, Tac^+) from large cell lymphoma. Proc Jpn Cancer Assoc 1986, 45:204
6. Kipps TJ, Herzenberg LA: Schemata for the production of monoclonal antibody-producing hybridomas, Handbook of Experimental Immunology. Vol. 4: Applications of Immunological Methods in Biomedical Sciences. Edited by DM Weir,

LA Herzenberg, C Blackwell, LA Herzenberg. Oxford, Blackwell Scientific Publications, 1986, pp 108.1–108.9
7. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature 1970, 227: 680–685
8. Towbin H, Staelhelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci U.S.A. 1979, 76:4350–4354
9. Lane HC, Whalen G, Fanci AS: In vitro evaluation of human lymphocyte function, Handbook of experimental immunology, Cellular Immunology. Edited by M Weir, LA Herzenberg, C Blackwell, LA Herzenberg. Oxford, Blackwell Scientific Publications, 1986, pp 66.1–66.14
10. Hsu SM, Raine L, Fanger H: The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
11. Suchi T, Tajima K, Nanba K, Wakasa H, Mikata A, Kikuchi M, Mori S, Watanabe S, Mohri N, Shamoto M, Harigaya K, Itagaki T, Matsuda M, Kirino Y, Takagi K, Fukunaga S: Some problems on the histopathological diagnosis of non-Hodgkin's malignant lymphoma: A proposal of a new type. Acta Pathol Jpn 1979, 29:755–776
12. Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T cell subsets in human lymph nodes. J Exp Med 1981, 153:30–41
13. Schuurman HJ, Van Baarlen J, Huppes W, Lam BW, Verdonck LF, Van Unnik JAM: Immunophenotyping of non-Hodgkin's Lymphoma: Lack of correlation between immunophenotype and cell morphology. Am J Pathol 1987, 129: 140–151
14. Broder S, Uchiyama T, Waldmann TA: Current concepts in immunoregulatory T-cell neoplasms. Cancer Treat Rep, 1979, 63:607–612
15. Picker LJ, Weiss LM, Medeiros LJ, Wood GS, Warnke RA: Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. Am J Pathol 1987, 128:181–201
16. Salter DM, Krajewski AS, Dewar AE: Immunohistochemical staining of non-Hodgkin's lymphoma with monoclonal antibodies specific for the leukocyte common antigen. J Pathol 1985, 146:345–353
17. Norton AJ, Isaacson PG: Detailed phenotypic analysis of B-cell lymphoma using a panel of antibodies reactive in routinely fixed wax-embedded tissue. Am J Pathol 1987, 128: 225–240
18. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PCL: Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL 1. Immunology 1986, 58: 63–70
19. West KP, Warford A, Fray L, Allen M, Campbell AC, Lauder I: The demonstration of B-cell, T-cell and myeloid antigens in paraffin sections. J Pathol 1986, 150:89–101

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