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S100-positive Histiocytes in T-Cell-dependent Area in Human Lymph Nodes Express P-Glycoprotein

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In the present study, we examined P-glycoprotein (P-gp)-expressing cells in human lymph nodes (LN) from patients with reactive lymph adenitis and various malignant lymphomas (ML). MDR1 mRNA was detected in all reactive LN and in 8 of 10 ML samples examined by reverse transcription polymerase chain reaction (RT-PCR). The signal intensity of the bands was stronger in samples from T-cell ML LN than in those from LN of reactive lymph adenitis and B-cell ML. Immunohistochemistry also showed positive reactivity with cellular components in the LN. The P-gp-positive cells, detected by UIC2, in reactive lymph adenitis and ML samples were large in size in the T-cell-dependent paracortical area in LN. Further, morphological characteristics were similar in UIC2-positive cells between reactive and ML LN. Double staining with UIC2 and a hematopoietic cell lineage-specific monoclonal antibody showed that UIC2-positive cell co-expressed S-100, but not other lymphocyte- and ML cell-specific antigens, indicating that these cells correspond to histiocytes in the T-cell-dependent paracortical area. Our results show that P-gp was expressed in histiocytes but not ML cells in LN, and suggest that this molecule may play an important role in the biological function of histiocytes and/or for maintenance of homeostatic levels in T-cell ML LN.

Key words: P-glycoprotein — MDR1 — Histiocyte — Normal lymph node — Malignant lymphoma

P-Glycoprotein (P-gp) plays an important role in drug resistance in neoplastic cells. Its expression, however, has also been demonstrated in various normal organs; for example, liver, kidney, adrenal gland, colon, thyroid gland, brain, testes, etc.[1-4] Physiologically, P-gp is considered to be an efflux pump for tissues with secretory or barrier functions, and this function is thought to facilitate drug resistance in neoplastic cells.[5,6]

Several investigators have reported detecting P-gp in normal and neoplastic cells in the hematopoietic system including the lymph nodes (LN).[2,7-12] However, it is still obscure what kind of cells in normal and neoplastic LN express the P-gp. There were also several technical problems in previous reports; total RNA extracted from whole LN tissue was used, or high concentrations of most available anti-P-gp monoclonal antibodies (mAb) were needed to detect P-gp. Thus, whether P-gp-expressing cells exist in normal LN remains controversial. In the present study, we investigated the character of P-gp-expressing cells in normal and malignant lymphoma (ML) LN using molecular genetic and immunohistochemical techniques to determine the phenotype of the P-gp-expressing cells in LN.

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MATERIALS AND METHODS

Patients and lymph nodes We studied fresh tissues from 12 patients with LN swelling. Twelve biopsy specimens were taken; two from reactive lymph adenitis with rheumatoid arthritis, and ten from ML. By means of immunophenotypical methods, the ten ML samples were classified into one adult T cell leukemia/lymphoma (ATLL), four B-cell non-Hodgkin's lymphoma (B-NHL), three T-cell non-Hodgkin's lymphoma (T-NHL), one follicular lymphoma (FL) and one Hodgkin's lymphoma (HD). Histological diagnosis was carried out according to the modified Working Formulation (Table I).

RT-PCR study Total RNA was extracted from tissue samples and cells separated as previously described.[13] MDR1 gene transcript was detected by the reverse transcription polymerase chain reaction method (RT-PCR).[14] A pair of 21-mer oligonucleotide primers was synthesized for RT-PCR of the human MDR1 gene: a primer corresponding to nucleotides 761 to 780 was used as the sense primer, and from 1333 to 1314 as the antisense primer.[15] The length of the RT-PCR product is 573 base pairs. After RT, 3 μg of the cDNA was subjected to 30 cycles of PCR, comprising a denaturation step for 1 min 30 s at 94°C, annealing for 2 min at 60°C, and extension for 2 min at 72°C. The PCR products were

Table I. Classification of Lymph Node Swelling Samples

Case	Diagnosis	Histology[a]
1	Reactive lymphadenitis	
2	Reactive lymphadenitis	
3	Adult T cell leukemia lymphoma	
4	B-NHL	Diffuse large cell
5	B-NHL	Diffuse large cell
6	B-NHL	Diffuse large cell
7	B-NHL	Diffuse large cell
8	T-NHL	Diffuse mixed cell
9	T-NHL	Diffuse mixed cell
10	T-NHL	Immunoblastic polymorphous
11	FL	Follicular small cleaved cell
12	HD	

a) Histology is given according to the Working Formulation: B-NHL=B cell non-Hodgkin's lymphoma, T-NHL=T cell non-Hodgkin's lymphoma, FL=follicular lymphoma, HD=Hodgkin's lymphoma.

visualized by ethidium bromide staining after agarose gel electrophoresis.

The RT-PCR products after electrophoresis were transferred to nitorocellulose membranes, and hybridized with MDR1 cDNA probe. The MDR1 cDNA was kindly provided by Dr. I. B. Roninson (University of Illinois, Chicago, IL).

Immunohistochemistry Cryostat sections (6 μm) of LN tissue were used for immunohistochemistry. The sections were fixed in 4% paraformaldehyde in ethanol at 4°C for 8 min and in ethanol at −20°C for 2 h, and then air-dried at room temperature for 30 min. After rinsing with 5% horse serum for 20 min, the sections were incubated with 1 or 2.5 μg/ml or UIC2, or an appropriate concentration of another mAb or polyclonal antibody (pAb). MRK-16 was used at a concentration of 10 μg/ml. The reaction products were visualized by the alkaline phosphatase anti-alkaline phosphatase method (APAAP), using biotin-labeled horse anti-mouse Ab (Vector Labs, Burlingame, CA), AP-conjugated avidin (Dakopatts, Glostrup, Denmark), and AP substrate kit (Vector). In addition, double immunohistochemistry using a mixture of a mAb and a pAb was performed. Before double immunostaining, endogenous peroxidase activity was blocked with 3% H_2O_2. Sections were incubated with a mouse mAb for 2 h after rinsing for 20 min in 5% horse serum, and were then washed 3 times with phosphate-buffered saline (PBS). The reaction products were visualized by APAAP. Sections were incubated with the other mAb or pAb for 1 h, after rinsing for 20 min with 5% rabbit serum, and were then washed 3 times in PBS and incubated with rabbit anti-mouse Ig for 30 min. The reaction products were visualized with diaminobenzidine and 0.01% H_2O_2.

The mouse anti-human P-gp mAbs, UIC2[6] and MRK16[16] were kindly provided by Drs. I. B. Roninson

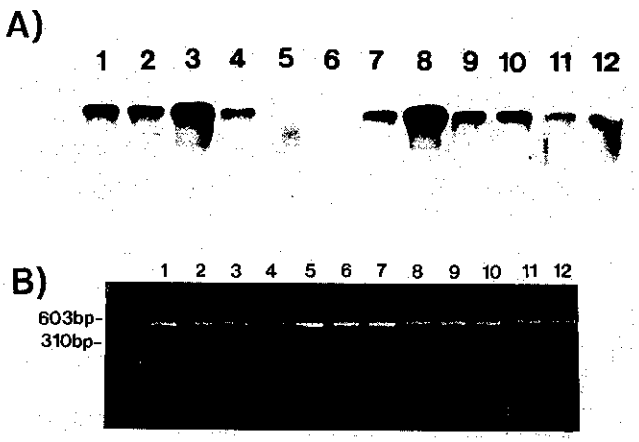

Fig. 1. MDR1 gene (A) and β-actin (B) expression determined by RT-PCR. A definitive band of 573 bp (MDR1 cDNA by RT-PCR) was detected in both reactive lymph adenitis samples (case No. 1 and 2) and all T-NHL samples (case No. 3, 8, 9 and 10), while a weak band was observed in two of four B-NHL samples (case No. 4 and 7), one FL sample (case No. 11) and one HD sample (case No. 12). In two B-NHL samples (case No. 5 and 6) MDR1 mRNA was not detected. Lane numbers are the same as case numbers in Table I.

(University of Illinois) and T. Tsuruo (University of Tokyo, Tokyo), respectively, CD2 (recognized by T11 mAb), CD5 (by Leu1), CD7 (by TP40), CD3 (by Leu4), CD4 (by Leu3a), and CD8 (by Leu2) were used as T-cell markers; CD16 (by Leu11) and CD57 (by Leu7) as natural killer cell markers; CD19 (by Leu12) and CD20 (by Leu16) as B-cell markers; CD21 (by CR2) as a follicular dendritic cell marker; CD1a (by Leu6) and S-100 as histiocyte in T-cell-dependent paracortical area markers[17, 18]; and CD11b (by OKM1)

and CD13 (by MCS2) as myeloid cell markers. Rabbit anti-lysozyme polyclonal Ab (pAb), rabbit anti-S100 pAb, anti-S100 mAb, and anti-myeloperoxidase (MPO) mAbs were from Dakopatts.

RESULTS

MDR1 gene expression Total RNA from LN was analyzed by RT-PCR using 2 samples from normal reactive LN and 10 samples with various types of ML (Table I). MDR1 gene expression was detected in both normal LN samples and in 8 of the 10 ML samples (Fig. 1). Also, the signal intensity was stronger in RNA samples from T-NHL LN (case No. 3, 8, 9 and 10) than in those from B-NHL LN (case No. 4, 5, 6, 7 and 11). On the other hand, the signal intensity of the Hodgkin's lymphoma sample was very low. The level of MDR1 transcript in samples from normal LN was lower than in samples from T-NHL, but was higher than in samples from B-NHL, FL and HD. The same RT products were subjected to PCR using β-actin primers, and the results showed them to contain almost the same amounts of RNA.

Immunohistochemistry In normal LN samples, UIC2-positive cells were distributed in the T-cell-dependent paracortical area and around the sinus area (Fig. 2A). UIC2-positive cells were large in size (Fig. 2B) and were found diffusely throughout the LN in both B-NHL and in T-NHL (Fig. 2C and 2D). No morphological differences were observed in UIC2-positive cells between

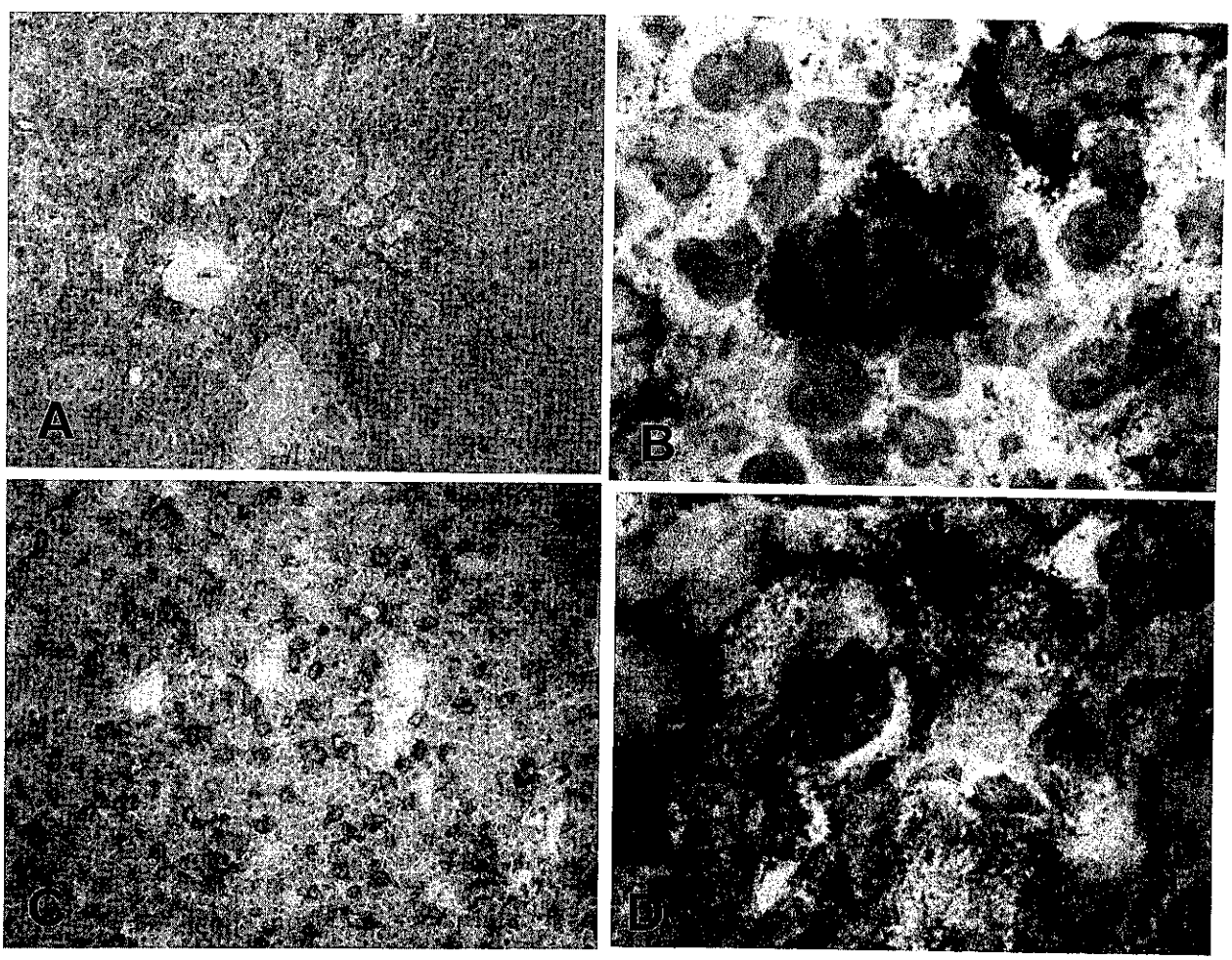

Fig. 2. Immunohistochemistry using UIC2. Reactive lymph node (A and B), and T-cell lymphoma (C and D). A and C, low magnification (×200); B and D, high magnification (×1000). A and B: Case 1. The UIC2-positive cells were seen in the T-cell dependent paracortical area and around a sinus area. C and D: Case 8. UIC2 reacted positively with large-sized cells showing no morphological abnormality in T-NHL.

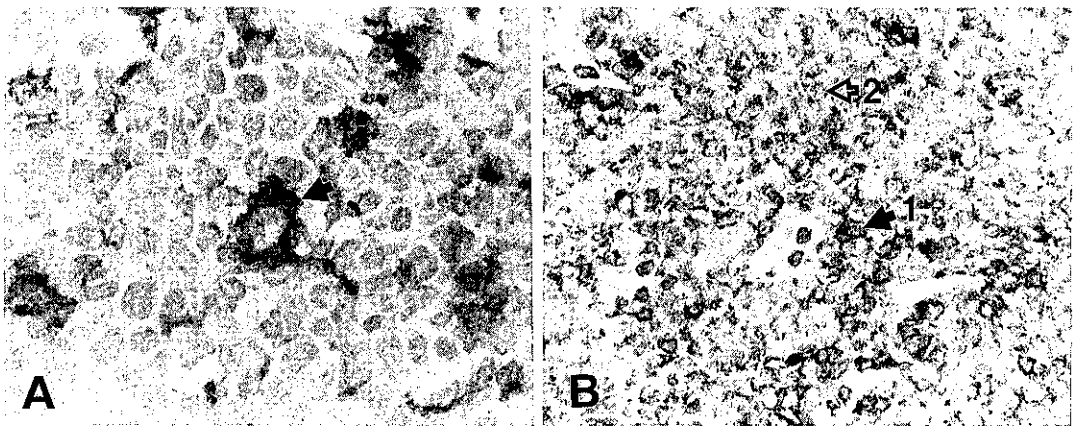

Fig. 3. Double staining of P-glycoprotein-positive cells. A and B: Case 8. (A) Double staining using UIC2 and anti-S-100. The arrow indicates a cell co-expressing P-glycoprotein and S-100. (B) Double staining using UIC2 and anti-CD3. Arrows 1 and 2 indicate P-glycoprotein- (red) and CD3- (brown) positive cells, respectively. No CD3-positive cells also reacted with UIC2.

normal, B-NHL, and T-NHL LN. The results using UIC2 were identical with those using MRK16. Here, the results of RT-PCR at 30 cycles corresponded well to those of immunohistochemistry.

Doubly stained sections showed that the large-sized UIC2-positive cells co-expressed S-100 (Fig. 3A), but did not express lysozyme or MPO. Here, P-gp-negative and S-100-positive cells were less than 10%, as previously described. Lymphocyte-associated antigens were detected only in UIC2-negative cells. In T-NHL LN, the UIC2-negative cells expressed one or more T lymphocyte-associated antigens with marked morphological abnormalities (Fig. 3B). Also proliferation of histiocytes was more frequently found in T-NHL LN, and the number of histiocytes corresponded well to the intensity of the MDR1 RT-PCR band. The two MDR-1 mRNA-negative cases (case No. 5 and 6) were not shown to have UIC2-positive cells. In this tissue, some vascular endothelial cells appeared to be stained very weakly by UIC2, although we could not exclude the possibility of this being a false-positive reaction.

DISCUSSION

Previous reports have clarified that P-gp- and MDR1 mRNA-positive cells exist in normal and neoplastic LN.[2, 8-10, 19, 20] Indeed, MDR1 gene transcripts were detected in RNA samples from both normal and neoplastic LN samples in this study. However, it remains unclear what kind of cells expressed P-gp in these tissues, because it is not easy to detect P-gp-positive cells in hematopoietic tissues by conventional fixation procedures. In this study, we used 4% paraformaldehyde/ethanol-fixed lymph nodes for immunohistochemistry with UIC-2 and MRK-16 antibodies. The results of immunohistochemistry corresponded well to those of RT-PCR.

Interestingly, the majority of P-gp-positive cells were distributed in the T-cell-dependent paracortical area in both normal and ML LN. These cells were characteristically large in size and co-expressed S-100, and were negative for both lysozyme and MPO. The morphological and phenotypic features of the P-gp-positive cells correspond to those of histiocytes in the T-cell-dependent paracortical area.[17, 18, 21]

The function of P-gp on the histiocytes remains to be determined. The P-gp expression may be related to the secretion of several cytokines from the histiocyte, as in adrenocortical cells.[22] Also, one physiological function of P-gp is thought to be in the maintenance of homeostatic levels in various organs.[23] Since P-gp expression on the histiocytes was more prominent in T-NHL, the P-gp expression may be induced by environmental changes according to excessive proliferation or infiltration of neoplastic cells in the LN.

Finally, several reports have described the expression of P-gp on a part of peripheral lymphocytes, as detected by dye-efflux test and indirect immunofluorescence using high concentrations of anti-P-gp antibodies.[24, 25] The possibility that small amounts of P-gp are expressed on lymphocytic components can not be ruled out. In this study, there were no significant differences between normal and neoplastic LN samples in the signals of MDR1 mRNA by RT-PCR, and the P-gp expression on both normal and neoplastic lymphocytes in the LN samples was below the sensitivity of the immunohistochemistry. We could not identify prominent or aberrant expression of P-gp on any type of lymphoma cells examined.

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