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Abnormal Expression of Blood Group-related Antigens in Uterine Endometrial Cancers

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The expression of A, B, and H group antigens, Lewis group antigens (Lewis^a, Lewis^b, Lewis^x, and Lewis^y), and Lc4 and nLc4 antigens, the precursor antigens of both groups, was examined immunohistochemically with monoclonal antibodies in 9 normal endometria, 6 endometrial hyperplasias, and 31 endometrial cancers. 1) A, B and/or H antigens were detected in endometrial cancers at an incidence of 51.6%, while no distinct localization of these antigens was observed in normal endometria. H antigen, the precursor of A and B antigens, was particularly frequently detected in endometerial cancers. 2) An increased rate of expression of Lewis group antigens, particularly Lewis^b antigen, was observed in endometrial cancers compared with its expression in normal endometria. 3) Lc4 and nLc4 antigens were detected in endometrial cancers at rates of 41.9% and 38.7%, respectively, these expressions being increased compared with those in normal endometria. 4) These results suggest that a highly abnormal expression of blood group-related antigens in endometrial cancers occurs not only at the level of A, B, and H antigens and Lewis group antigens, but also at the level of their precursor Lc4 and nLc4 antigens. 5) Lewis^a, Lewis^b, and Lc4 antigens, built on the type-1 chain, are more specific to endometrial cancers than their respective positional isomers, Lewis^x, Lewis^y, and nLc4 antigens, built on the type-2 chain.

Key words: Blood group-related antigens — Lc4, nLc4 — Monoclonal antibody — Immunohistochemistry — Endometrial cancer

In Japan, uterine endometrial cancers are gradually increasing not only in frequency but also in absolute number. However, since the nuclear atypia of endometrial cancer cells is often inconspicuous, cytologic diagnosis is sometimes difficult, especially in the well-differentiated type. Therefore, it is necessary to define cell biological characteristics of endometrial cancers that can be applied diagnostically as a supplement to morphologic diagnosis.[1)]

Blood group-related carbohydrate antigens are important human alloantigens that have been studied extensively. It has been demonstrated that changes in their phenotypic expression can occur during the processes of normal development[2)] and neoplastic transformation.[3)] In addition, many monoclonal antibodies raised against human cancer cells have been shown to react with these carbohydrate structures, for example, CA19-9.[4)] Thus, investigation of the abnormal expression of blood group-related antigens in cancer is very useful for shedding light on the mechanism of changes in carbohydrate antigens in association with neoplastic transformation.[5)]

Recently, abnormal expression of blood-related carbohydrate antigens in endometrial cancers has been reported as a result of immunohistochemical application of newly generated monoclonal antibodies.[6–8)] However, there have been few reports on the expression of lactotetraosyl ceramide (Lc4) and lactoneotetraosyl ceramide (nLc4), the precursor carbohydrates of A, B, and H group antigens and Lewis group antigens. Such studies may be helpful to obtain more complete knowledge regarding the mechanism of changes in carbohydrate antigens.

In this investigation, we studied the expression of not only A, B, and H group antigens and Lewis group antigens, but also Lc4 and nLc4 antigens in normal endometria, endometrial hyperplasias, and endometrial cancers.

MATERIALS AND METHODS

Immunohistochemistry using monoclonal antibodies against carbohydrates was applied to formalin-fixed, paraffin-embedded sections of surgical materials. Nine cases of normal endometrium (4 in the proliferative phase and 5 in the secretory phase), 6 cases of endometrial hyperplasia (3 adenomatous hyperplasia and 3 atypical hyperplasia cases), and 31 cases of endometrial cancer (19 well-differentiated, 6 moderately differen-

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tiated, and 6 poorly differentiated cases) were studied in this investigation. Benign materials were obtained from noncancerous uteri.

Mouse monoclonal antibodies with specificities for A, B and H group antigens were purchased from DAKO Corporation, while monoclonals against Lewis^a and Lewis^b antigens were obtained from Green Cross Corporation. Monoclonals against so-called Lewis^x and Lewis^y antigens[9] (below, they are described simply as Lewis^x and Lewis^y antigens) were provided by Dr. Hirohashi (Pathology Division, National Cancer Center Research Institute, Tokyo), and a monoclonal against nLc4 antigen,[10] which reacts with the nonreducing terminal structure of type-2 chain (Galβ1-4GlcNAc), was donated by Dr. Hata (Department of Pathology, Keio University). A hybridoma producing human monoclonal antibody against Lc4 antigen (HMST-1) was established in our laboratory.[11] The epitope recognized by HMST-1 was determined to be a lacto-series type-1 chain containing glycosphingolipid (Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer).

Immunohistochemical staining was performed by the avidin-biotin complex method. Tissue sections were incubated with monoclonal antibody at room temperature for one hour and subsequently treated with biotinylated horse anti-mouse IgG and avidin-biotin complex reagent (Vector Laboratory Inc., Burlingame, CA). Anti-A, -B, and -H monoclonals were used at a dilution of 1:50, whereas anti-Lewis^a, anti-Lewis^b, anti-Lewis^x, and anti-Lewis^y ones were diluted 1:100 with PBS. Anti-nLc4 was applied as an undiluted hybridoma culture supernatant, and the working concentration of anti-Lc4 was adjusted to 10 μg/ml. Sections were developed with diaminobenzidine for color and counterstained with hematoxylin. The staining patterns of each endometrial specimen were evaluated on the basis of both staining intensity and incidence of positive cells. The intensity was graded on an arbitrary scale as weak, moderate, or strong; and the incidence was classified into three grades of $<10\%$, 10% to 50%, and $>50\%$ according to the percentage of positive endometrial cells in each section. By combining both the intensity and incidence, we classified the reactivity of each specimen as ($\pm$), (+), (++), or (+++). The cases in the ($\pm$) group were handled as negative so that the results would be reliable. The specificity of immunohistochemical staining was confirmed by means of negative control sections in which the primary monoclonal antibodies were replaced by other mouse antibodies. Erythrocytes and vascular endothelial cells in the sections served as useful internal positive controls for the specificity of anti-A, -B, and -H antibodies.

RESULTS

Normal endometrium None of the specimens of normal endometrium distinctly expressed any of the A, B, and H blood group antigens, although these antigens were detected faintly on the luminal surface of normal endometrial glands in some cases (Fig. 1). With regard to the Lewis group antigens, no distinct localization of Lewis^b antigen was observed in the 9 normal endometria, but Lewis^a, Lewis^x, and Lewis^y antigens were detected in 2 [1 case each of (+) and (++)], 4 [1 case (+), 2 cases (++), and 1 case (+++)], and 2 [1 case each of (+) and (++)], respectively, of the 9 cases. Lc4 antigen and nLc4 antigen were also expressed in 1 [(+) case] and 1 [(++) case], respectively, of the 9 cases (Fig. 2).

Endometrial hyperplasia Concerning the expression of A, B and/or H antigens in the 6 cases of endometrial hyperplasia (3 adenomatous hyperplasia and 3 atypical hyperplasia cases), compatible antigens were observed in 1 case (+) of adenomatous hyperplasia (Fig. 3) and 1 case (++) of atypical hyperplasia. Lewis^a antigen was observed in 1 case of adenomatous hyperplasia [(++) case] and in 2 of the 3 atypical hyperplasias [1 case each of (+) and (++)]. Lewis^b antigen was observed in 1 case of adenomatous hyperplasia [(++) case] and 2 cases of atypical hyperplasia [1 case each of (++) and (+++)]. Lewis^x antigen was detected in 1 case of adenomatous hyperplasia [(+) case] and in all 3 cases of atypical hyperplasia [2 cases (++) and 1 case (+++)].

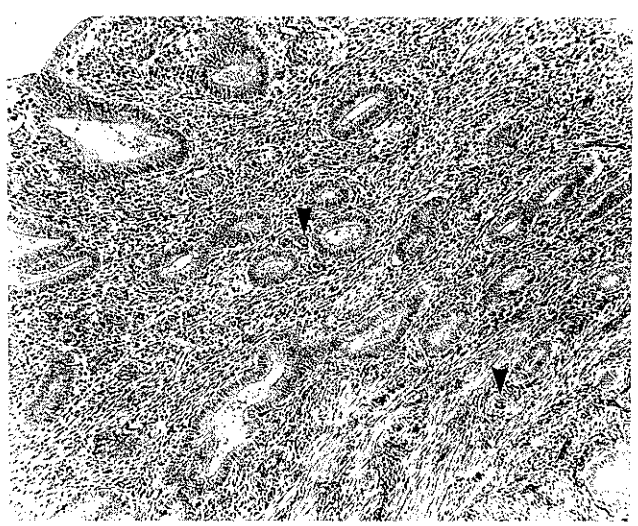

Fig. 1. Distribution of A antigen in normal proliferative endometrium. In contrast to the expression of A antigen in vascular endothelial cells and erythrocytes (indicated by arrows), endometrial cells are negative (original magnification, $\times$100).

Table I. Incidence of Endometrial Cancers Classified by the Expression Pattern of A, B, and H Blood Group Antigens and Blood Group of Patients

Type	Expression pattern of cancer	Blood group of patients				No. of cases (%)
		A	B	O	AB	
I	No blood group antigen	3	6	5	1	15 (48)
II	Existence of the precursor H blood antigen	2	2	/	1	5 (16)
III	Co-existence of the compatible and precursor H blood antigen	3	0	0	0	3 (10)
IV	Existence of the compatible antigen only	1	0	5	2	8 (26)
	Total	9	8	10	4	31 (100)

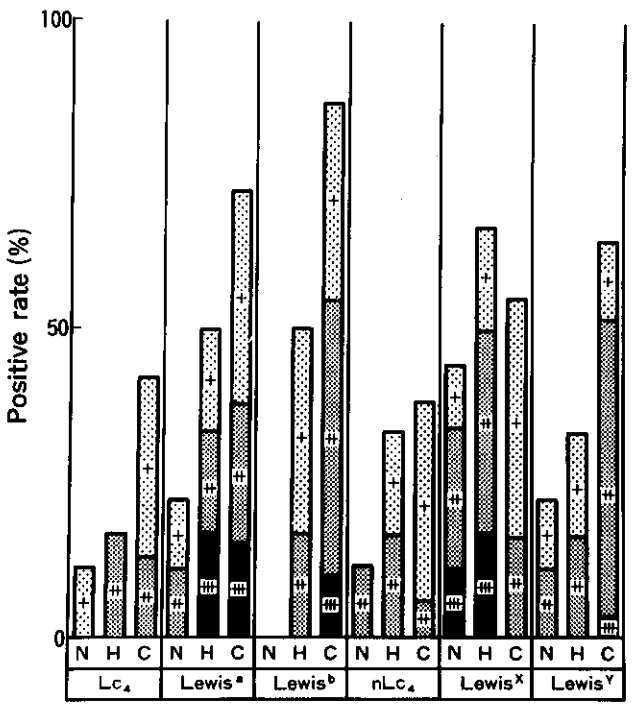

Fig. 2. Frequency of detection of type-1 chain (Lc4, Lewis^a, Lewis^b) and type-2 chain (nLc4, Lewis^x, Lewis^y) antigens in various kinds of endometrial lesions. N, normal endometrium; H, endometrial hyperplasia; C, endometrial cancer.

Lewis^y antigen was detected in only 2 cases of atypical hyperplasia [1 case each of (++) and (+++)]. Lc4 antigen and nLc4 antigen were not detected in the adenomatous hyperplasias, but they were detected in 1 case of atypical hyperplasia, in which they were moderately positive (Fig. 2).

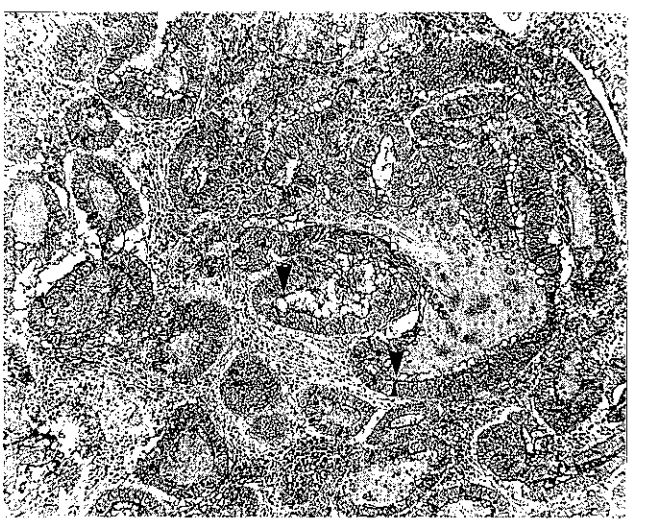

Fig. 3. Distribution of H antigen in adenomatous hyperplasia. Both the luminal surface and the cytoplasm of hyperplastic cells are moderately stained, as indicated by arrows (original magnification, ×100).

Endometrial cancer Concerning A, B and/or H antigens, not only compatible antigens but also their precursor antigens were detected in endometrial cancers, and the expression pattern could be classified into 4 types (Table I). Type I: Endometrial cancers expressing no blood-group antigens, as in normal endometria. Fifteen cases belonged to this type, and the incidence was 48% (15/31). Type II: Endometrial cancers expressing the precursor H antigen. Five cases belonged to this type, with an incidence of 16% (5/31). Type III: Endometrial cancers expressing both compatible and precursor H antigens (Fig. 4). Three cases belonged to this type, and

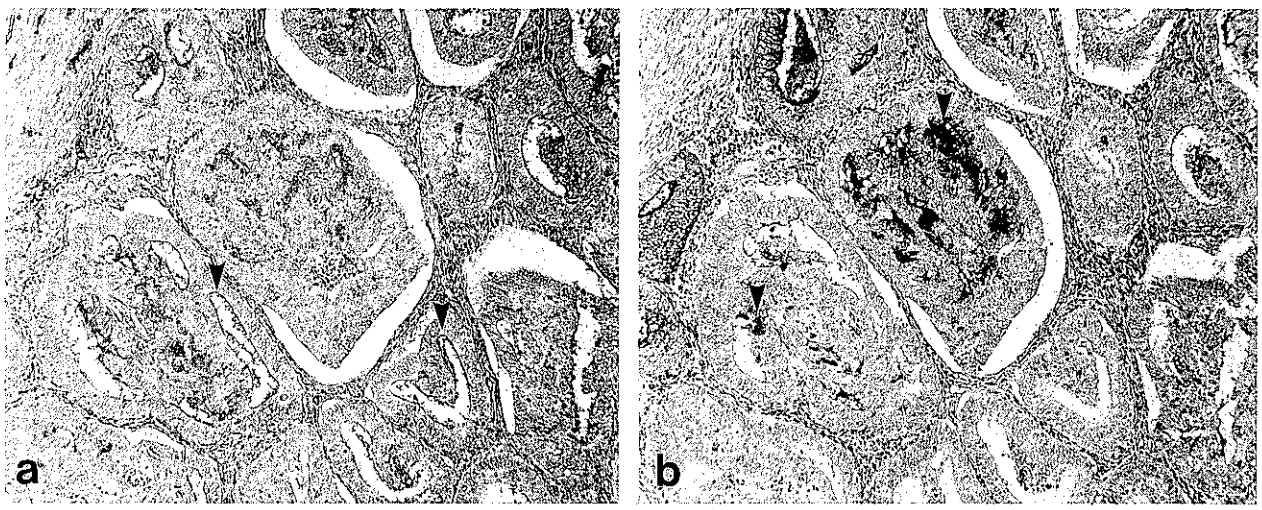

Fig. 4. Distribution of A (a) and H (b) antigens in consecutive sections of an endometrial cancer specimen of a blood group A patient. Not only A antigen (the compatible antigen for the patient) but also H antigen (the precursor of A antigen) is strongly stained in the endometrial cancer cells (indicated by arrows). This case was therefore classified as Type III (original magnification, ×100).

Table II. Relationships of A, B, and H Blood Group Antigens Expressed in Endometrial Cancers to Blood Group of the Patients

Antigens expressed in cancers	Blood group of patients				No. of cancers (Total 31)
	A (9)	B (8)	O (10)	AB (4)	
A	4	0	0	2	6
B	0	0	0	0	0
H	5	2	5	1	13
—	3	6	5	1	15

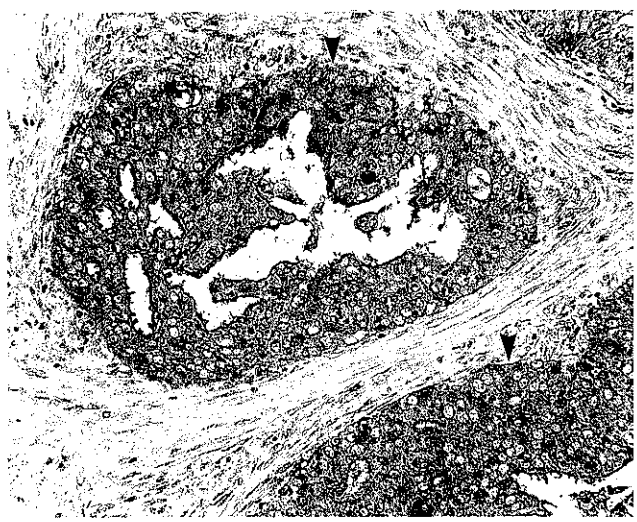

Fig. 5. Distribution of Lewis^b antigen in endometrial cancer. Almost all cancer cells are strongly positive (original magnification, ×200).

the incidence was 10% (3/31). Type IV: Endometrial cancers expressing only the compatible antigens. Eight cases belonged to this type, for an incidence of 26% (8/31).

Of the 31 endometrial cancer cases, 16 belonged to Type II, Type III, or Type IV, all types expressing A, B, and/or H antigens, and the incidence of expression was thus 51.6% (16/31), while no distinct localization of A, B, and H antigens was observed in normal endometria. As for the relationship between the expression of A, B, and H antigens in endometrial cancers and the blood group of the patients (Table II), H antigen was expressed on cancer cells from not only group O patients, but also group A, group B, and group AB cases. Its expression rate was 41.9% (13/31). The A group antigen was expressed in 6 of the 31 cases (19.4%) and only in group A and group AB patients in which the A antigen was the compatible antigen, with an expression rate of 46.2% (6/13); while the B group antigen was not expressed in any of the cancers. Further, endometrial cancers from group O patients expressed only H antigen or none, and

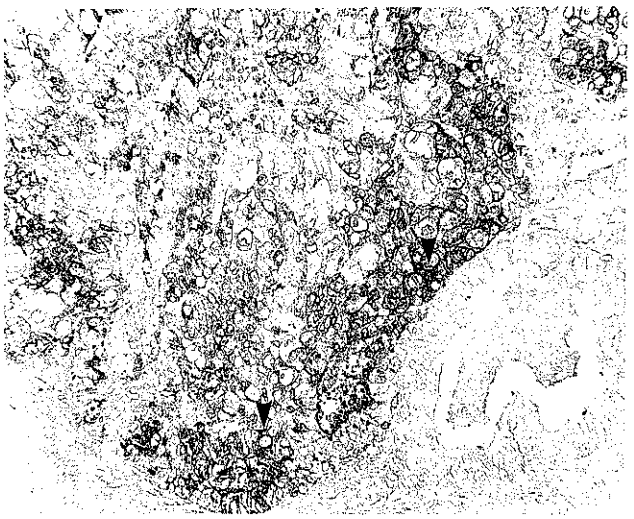

Fig. 6. Distribution of Lc4 antigen in endometrial cancer. Many cancer cells show intense staining of the membrane and cytoplasm, as indicated by arrows (original magnification, ×200).

neither A antigen nor B antigen was observed in these cancers. Endometrial cancers from group A patients expressed A antigen and/or H antigen, while those from group B patients expressed only the H group antigen. Thus, incompatible expression was not observed in any of the endometrial cancers studied.

With regard to Lewis group antigens, Lewis^a, Lewis^b, Lewis^x, and Lewis^y antigens were expressed in 23 cases (74.2%), 27 cases (87.1%), 17 cases (54.8%), and 20 cases (64.5%), respectively (Fig. 2). Therefore, the rate of expression of Lewis group antigens, particularly Lewis^b antigen (Fig. 5), was increased in endometrial cancers compared with that in normal endometria. Further, Lc4 antigen and nLc4 antigen were detected in 13 (41.9%) and 12 (38.7%), respectively, of the 31 endometrial cancers. In the 15 endometrial cancers belonging to Type I (not expressing A, B, or H antigens), 8 cases (53.3%) were positive for Lc4 antigen and 6 cases (40%) were positive for nLc4 antigen. The expression of Lc4 (Fig. 6) and nLc4 in endometrial cancers was thus increased compared with that in normal endometria.

Comparison of the immunohistochemical reactivity between each set of positional isomers in the same endometrial cancers (Fig. 7) Lewis^a antigen and Lewis^x antigen (a), both of which are fucosylated at the penultimate GalNAc, Lewis^b antigen and Lewis^y antigen (b), both of which are fucosylated at the penultimate GalNAc and at the terminal galactose, and Lc4 antigen and nLc4 antigen (c), neither of which are fucosylated, were compared in the same endometrial cancers. The

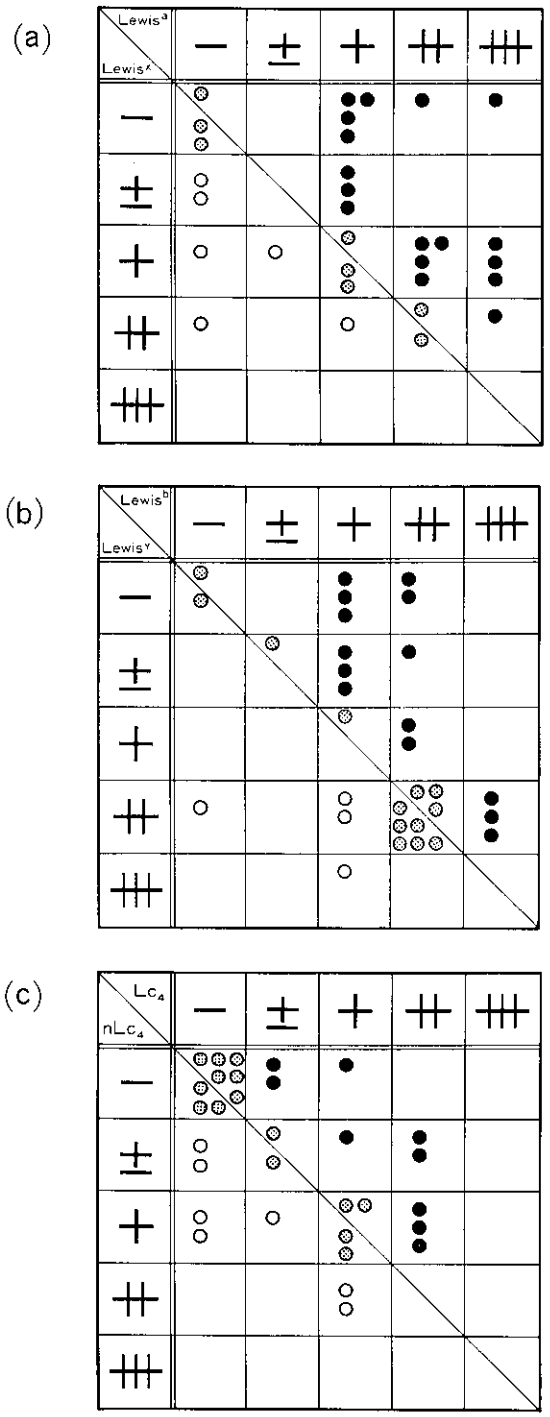

Fig. 7. Comparison of immunohistochemical reactivities between Lewis^a and Lewis^x (a), Lewis^b and Lewis^y (b), and Lc4 and nLc4 (c) in the same endometrial cancers. ●: The cases in which the reactivity of Le^a, Le^b, and Lc4 was stronger than that of Le^x, Le^y, and nLc4. ○: The cases in which the reactivity of Le^a, Le^b, and Lc4 was equal to that of Le^x, Le^y, and nLc4. ○: The cases in which the reactivity of Le^a, Le^b, and Lc4 was weaker than that of Le^x, Le^y, and nLc4.

cases in which the reactivity of Lewis^a antigen, Lewis^b antigen, and Lc4 antigen, built on the type-1 chain, was stronger than that of Lewis^x antigen, Lewis^y antigen and nLc4 antigen, built on the type-2 chain, amounted to 17 (54.8%), 14 (45.2%), and 9 (29.0%), respectively, of the 31 cases. The cases in which the reactivity of Lewis^a antigen, Lewis^b antigen, and Lc4 antigen was equal to that of Lewis^x antigen, Lewis^y antigen, and nLc4 antigen were 8 (25.8%), 13 (41.9%), and 15 (48.4%), respectively. Finally, cases in which the reactivity of Lewis^a antigen, Lewis^b antigen, and Lc4 antigen was weaker than that of Lewis^x antigen, Lewis^y, antigen and nLc4 antigen were 6 (19.4%), 4 (12.9%) and 7 (22.6%), respectively.

Relationship between blood group-related antigens and hitologic differentiation (Fig. 8) At least one of the A, B, and H antigens was expressed in 13 of the 19 well-differentiated cases (68.4%), 2 of the 6 moderately differentiated cancers (33.3%), and 1 of the 6 poorly differentiated cases (16.7%). As regards the Lewis group antigens, either Lewis^a antigen or Lewis^b antigen and either Lewis^x antigen or Lewis^y antigen was expressed in 18 (94.7%) and 17 (84.2%) of the 19 well-differentiated cases, 6 (100%) and 5 (83.3%) of the 6 moderately differentiated cases, and 4 (66.6%) and 2 (33.3%) of the 6 poorly differentiated cases, respectively. Lc4 antigen and nLc4 antigen were positive in 6 (31.6%) and 7 (36.8%) of the 19 well-differentiated cancers, 4 (66.6%) and 3 (50%) of the 6 moderately differentiated cancers, and 3 (50%) and 2 (33.3%) of the 6 poorly differentiated cancers, respectively.

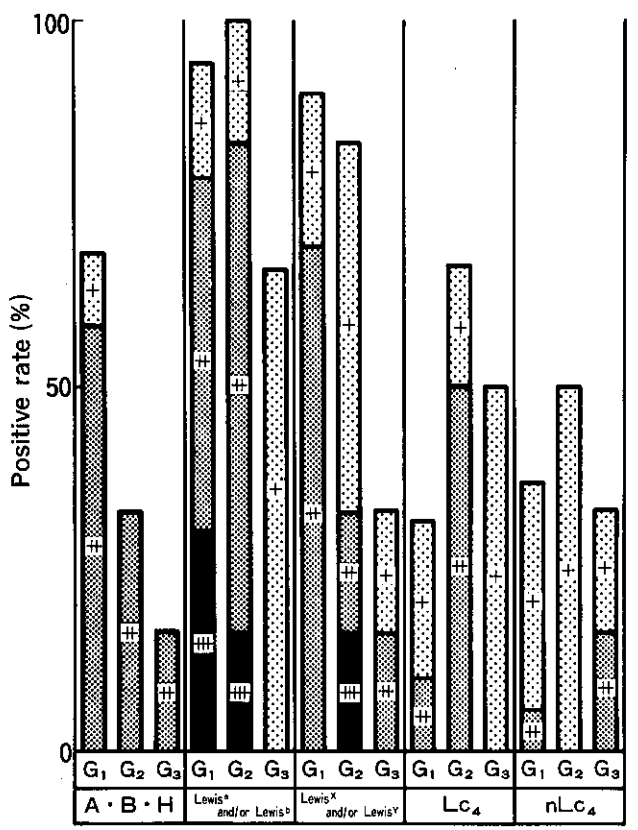

Fig. 8. Relationship of blood group-related antigens expressed in endometrial cancers to histologic differentiation. G1, well-differentiated; G 2, moderately differentiated; G 3, poorly differentiated.

DISCUSSION

Comparative studies on the expression of blood group-related antigens in normal and cancer tissues have been performed for many organs, such as the stomach,[12] lung,[13] colon,[14] breast,[15] urinary bladder,[16] uterus,[6, 7, 17] etc. According to those reports, A, B, and H antigens compatible with the blood group of the patients were found in the normal tissues of the stomach, lung, breast, urinary bladder, and uterine cervix in nearly all cases. Moreover, a tendency toward deletion of A, B and/or H antigens after neoplastic transformation is recognizable and may be understood as a dedifferentiation in association with neoplastic transformation. On the other hand, the reverse tendency was also observed: A, B, and H antigens are not found in the normal distal colon,[14] and yet they are expressed in association with cancer. A recent study based on the use of monoclonal antibodies demonstrated expression of A, B, and/or H antigens in endometrial cancers, whereas those antigens are not observed in the normal endometrium.[7] These observations strongly favor the possibility that abnormal expression of blood group-related carbohydrate antigens similar to that in the distal colon might occur in endometrial cancers. The mechanism of such abnormal expression of blood group-related carbohydrate antigens has not been clarified, but various concepts have been considered such as "accumulation of precursor carbohydrates dependent on a lack or deficiency of glycosyltransferase activities," "sialylation and polyfucosylation of antigens," "neo-expression of antigens, including incompatible antigens," etc.[5, 15, 16, 18] However, except for the expression of incompatible antigens, the above concepts are applicable only to cancers such as lung cancer and breast cancer, which lose A, B, and/or H antigens, but not to cancers such as distal colon cancer or endometrial cancer, which express those antigens in association with neoplastic transformation. Thus, new concepts for the latter should be considered. Regarding the blood group-related carbohydrate antigens, it is a well-known fact that these structures are formed by the sequential addition of monosaccharide units to the carbohydrate side chains of glycolipids or

glycoproteins. Accordingly, as the mechanism by which A, B, and/or H antigens are expressed in endometrial cancers, the concept of promotion of glycosyltransferase activity during carcinogenesis is plausible. That is, fucosyl transferase, GalNAc transferase, and/or Gal transferase may somehow be activated during carcinogenesis, resulting in the expression of H, A, and/or B antigens. Similarly, the increase in expression of Lc4 and nLc4 antigens in endometrial cancers may be explained by promotion of Gal transferase activity, which binds Gal to the terminal GlcNAc to form Lc4 or nLc4. Indeed, several investigators have reported that serum N-acetyl glucosaminyl glycoprotein β1-4 galactosyltransferase activity is elevated in ovarian cancer patients.[19, 20]

Although this hypothesis has not been confirmed, the possibility of removal of masking substances associated with the cancer process cannot be ruled out. That is, blood group-related antigens naturally existing on the surface of endometrial cells might be masked with some substance in a normal tissue, while in the cancerous state the masking substance would be lost and their antigenicity would thus be expressed. Indeed, Lc4 antigen, which is hardly expressed in normal endometrium, becomes detectable at a higher rate in normal endometrium treated with sialidase,[21] a rate comparable to that found for endometrial cancers in our present investigation. Therefore, further studies at the levels of glycosyltransferase and glycosidase are necessary to shed more light on the mechanism of abnormal expression of blood group-related antigens in uterine endometrial cancers.

With respect to the expression of Lewis group antigens, and Lc4 and nLc4 antigens in endometrial cancers, all antigens were detected at a higher rate in endometrial cancers than in normal endometrium, and this tendency was similar to that observed by staining for Lewis^a and Lewis^b antigens in the distal colon.[14] Among these antigens, Lewis^a, Lewis^b, and Lc4 antigens, built on the type-1 chain, showed higher specificity for endometrial cancers than their corresponding positional isomers, Lewis^x, Lewis^y and nLc4 antigens, built on the type-2 chain. These results are similar to those reported by Inoue *et al.*,[7] but are different from those reported by Kannagi *et al.*,[22] who examined gastrointestinal and lung cancers. Kannagi and co-workers found that the type-2 chain was 100 to 200 times more frequent in cancer tissues than in the corresponding normal tissues, which showed a predominance of type-1 chains over type-2 chains. The reason for this great discrepancy is not clear. Lewis^a and Lewis^x antigens are fucosylated at the penultimate GalNAc, and Lewis^b and Lewis^y antigens have additional fucosylation at the terminal galactose. Increased expression of Lewis^a, Lewis^b, Lewis^x, and Lewis^y antigens in endometrial cancers strongly suggests that fucosyltransferase activities might be promoted in endometrial cancers, considering the results for A, B, and H antigens.

We did not determine the secretor/non-secretor status of the patients included in this study. In general, the population can be divided into a major group of secretors (about 80%) and a minor group of non-secretors (about 20%). In the non-secretor, H antigen is not formed, and A and B antigen are also not synthesized. Moreover, Lewis^b antigen can not be formed.[12] With regard to endometrial cancer, A, B, and H antigen were not detected in 48.4% of the cases and Lewis^b antigen was not detected in 12.9% of the cases. Therefore, it cannot be ruled out that tissues from non-secretor patients might have been included in those cases. However, the blood group antigens are not expressed in the normal endometrium, but do appear in parallel with neoplastic transformation. Therefore, even if the secretor/non-secretor status of the patients is taken into consideration, it seems safe to assume that, in the secretor, the incidence of expression of A, B, or H antigen or Lewis^b antigen in endometrial cancers will not decrease compared with that indicated in this study.

Finally, in endometrial hyperplasia, the positive rates of A, B, and H antigens, Lewis group antigens, and Lc4 and nLc4 antigens were intermediate between those for the normal endometrium and those for endometrial cancers. The rates in atypical hyperplasia, presently classified as a borderline lesion or 0 stage of endometrial cancer, were higher than in benign adenomatous hyperplasia. These results suggest that changes in blood group-related antigens have already been induced in preneoplastic lesions, as Hirohashi *et al.* reported,[12] and increase as the lesions grow worse. This is very interesting when considering the changes in carbohydrate chain antigens accompanying neoplastic transformation.

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REFERENCES

1) Nozawa, S., Sakayori, M., Ohta, K., Iizuka, R., Mochizuki, H., Soma, M., Fujimoto, J., Hata, J., Iwamori, M. and Nagai, Y. A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry. *Am. J. Obstet. Gynecol.*, **161**, 1079–1086 (1981).
2) Feizi, T. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are oncodevelopmental antigens. *Nature*, **314**, 53–57 (1985).
3) Kavarik, S., Davidsohn, I. and Stejkal, R. ABO antigens in cancer. *Arch. Pathol.*, **86**, 12–21 (1968).
4) Magnani, J. L., Nilsson, B., Brockhaus, M., Zopt, D., Steplewski, Z., Koprowski, H. and Ginsburg, V. A monoclonal antibody defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J. Biol. Chem.*, **257**, 14365–14369 (1982).
5) Hakomori, S. Tumor associated carbohydrate antigens. *Ann. Rev. Immunol.*, **2**, 103–126 (1984).
6) Tsuji, Y., Yoshioka, M., Ogasawara, T., Takemura, T. and Isojima, S. Identification of an H antigen-like blood group antigen in sera of cancer patients using a novel monoclonal antibody raised against endometrial carcinoma. *Cancer Res.*, **47**, 3543–3550 (1987).
7) Inoue, M., Sasagawa, T., Saito, J., Shimizu, J., Ueda, G., Tanizawa, O. and Nakayama, M. Expression of blood group antigens A, B, H, Lewis^a, and Lewis^b in fetal, normal, and malignant tissue of the uterine endometrium. *Cancer*, **60**, 2985–2993 (1987).
8) Iwamori, M., Sakayori, M., Nozawa, S., Yamamoto, T., Yago, M., Noguchi, M. and Nagai, Y. Monoclonal antibody-defined antigen of human uterine endometrial carcinomas is Le^b. *J. Biochem.*, **105**, 718–722 (1989).
9) Watanabe, M., Hirohashi, S., Shimosato, Y., Ino, Y., Yamada, T., Teshima, S., Sekine, T. and Abe, O. Carbohydrate antigen defined by a monoclonal antibody raised against a gastric cancer xenograft. *Jpn. J. Cancer Res.*, **76**, 43–52 (1985).
10) Fujimoto, J., Hata, J., Ishii, E., Tanaka, R., Kannagi, R., Ueyama, Y. and Tamaoki, N. Differentiation antigens defined by mouse monoclonal antibodies against human germ cell tumors. *Lab. Invest.*, **57**, 350–358 (1987).
11) Nozawa, S., Narisawa, S., Kojima, K., Sakayori, M., Iizuka, R., Mochizuki, H., Yamauchi, T., Iwamori, M. and Nagai, Y. Human monoclonal antibody (HMST-1) against lacto-series types 1 chain and expression of the chain in uterine endometrial cancers. *Cancer Res.*, **49**, 6401–6406 (1989).
12) Hirohashi, S., Shimosato, Y., Ino, Y., Tome, Y., Watanabe, M., Hirota, T. and Itabashi, M. Distribution of blood group antigens and CA19-9 in gastric cancers and non-neoplastic gastric mucosa. *Gann*, **75**, 540–547 (1984).
13) Hirohashi, S., Ino, Y., Kodama, T. and Shimosato, Y. Distribution of blood group antigens A, B, H and I (Ma) in mucus-producing adenocarcinoma of human lung. *J. Natl. Cancer Inst.*, **72**, 1299–1305 (1984).
14) Yuan, M., Itzkowitz, S. H., Palekar, A., Shamsuddin, A. M., Phelps, P. C., Trump, B. F. and Kim, Y. S. Distribution of blood group antigens A, B, H, Lewis^a and Lewis^b in human normal, fetal and malignant colonic tissue. *Cancer Res.*, **45**, 4499–4511 (1985).
15) Lee, A. K., Delellis, R. A., Rosen, P. P., Saigo, P. E., Gangi, M. D., Bagin, R., Groshen, S. and Wolfe, H. J. ABH blood group isoantigen expression in breast carcinomas — an immunohistochemical evaluation using monoclonal antibodies. *Am. J. Clin. Pathol.*, **83**, 308–319 (1985).
16) Juhl, B. R., Hartzen, S. and Haina, B. A, B, H antigen expression in transitional cell carcinomas of the urinary bladder. *Cancer*, **57**, 1768–1775 (1986).
17) Sasagawa, T., Inoue, M., Shimizu, C., Shimizu, H., Saito, J., Ueda, G., Tanizawa, O. and Nakayama, M. Expression of blood group antigens A, B, H, Lewis^a, Lewis^b in malignant lesions of uterine cervix. *Acta Obstet. Gynaecol. Jpn.*, **40**, 345–352 (1988).
18) Clausen, H. and Hakomori, S. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. *Vox Sang.*, **56**, 1–20 (1989).
19) Bhattacharya, M., Chatterjee, S. K. and Barlow, J. J. Uridine 5′-diphosphate galactose:glycoprotein galactosyltransferase activity in the ovarian cancer patients. *Cancer Res.*, **36**, 2096–2101 (1976).
20) Goldhirsch, A., Berger, E., Muller, O., Maibach, R., Misteli, S., Buser, K., Roesler, H. and Brunner, K. Ovarian cancer and tumor markers: sialic acid, galactosyltransferase and CA-125. *Oncology*, **45**, 281–286 (1988).
21) Nozawa, S. Comprehensive basic study on the expression mechanism of some oncofetal antigens and their clinical application — from the production of monoclonal antibodies to *in vitro* analysis. *Acta Obstet. Gynaecol. Jpn.*, **39**, 1213–1220 (1987).
22) Kannagi, R. and Hakomori, S. Analysis of cancer-associated carbohydrate antigens with monoclonal antibodies. *Metab. Dis.*, **23**, 141–154 (1986) (in Japanese).