Virchows Archiv A Pathol Anat (1991) 418:157–162

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunodetection of sialyl-Tn antigen in normal, hyperplastic and cancerous tissues of the uterine endometrium

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Received October 12, 1990 / Accepted October 23, 1990

Summary. The expression of sialyl-Tn antigen (STn) in normal, hyperplastic and neoplastic tissues of the uterine endometrium was examined by immunoperoxidase staining of formalin-fixed, paraffin-embedded samples using the monoclonal antibody TKH-2, directed toward the STn structure (NeuAc 2-6GalNac 1-O-serine or threonine). STn was expressed in 13 of 18 normal postovulatory endometria with an increasing staining intensity and incidence in the late secretory phase. It was consistently absent in 10 proliferative endometria. None of 5 cystic, 4 adenomatous or 12 atypical hyperplasias expressed STn, but areas of severe cytological atypia in 3 atypical hyperplasias showed faint expression. STn expression was detected in 36 of 43 adenocarcinomas. Although the extent of staining varied from a few to most of the cancer cells, general staining was observed throughout the cytoplasm of cancer cells with increased staining of the luminal surface and frequent positive staining of intraluminal mucin. Thus, it is clear that STn is selectively expressed in cancer cells and shows restricted expression in normal and hyperplastic endometrial tissues. STn may be an early marker of malignant transformation and has potential for use as a diagnostic aid in the surgical pathology of the uterine endometrium.

Key words: Sialyl-Tn antigen – Endometrial hyperplasia – Mucin-type monosaccharide – Tumour marker – Immunohistochemistry

Introduction

Precancerous lesions of the endometrium have been recognized to include a broad spectrum of malignant transformations and have been investigated for many years. However, considerable uncertainty remains concerning the morphological diagnosis as well as the biological significance of these lesions. Most pathologists experience great difficulty in distinguishing precancerous lesions, especially atypical forms of hyperplasia, from well-differentiated adenocarcinoma (Welch and Scully 1977; Kurman and Norris 1982; Bhagavan et al. 1984). As a result, many attempts have been made to establish objective diagnostic criteria using quantitative cytomorphometry and cytophotometry. These techniques have achieved partial success in the laboratory but are very cumbersome in practical medicine (Baak et al. 1981a, b; Norris et al. 1989; Thornton et al. 1989).

Recent advances in hybridoma technology have facilitated the identification of novel antigens, monoclonal antibodies to some of which have demonstrated selective reactivity for malignant versus benign tissues. The sialyl-Tn (STn) antigen is one such tumour-associated antigen, the immunodeterminant of which is the sialylated form of a glycoprotein containing *N*-acetyl galactose connected by *O*-glycosidic linkages to serine or threonine residues in the protein backbone (Hakomori 1984). Recently, a monoclonal antibody (TKH-2) recognizing STn was generated using bovine submaxillary mucin as an immunogen, and immunohistochemical studies have demonstrated its reactivity with a large number of adenocarcinomas of the gastrointestinal tract, while it does not react with normal tissues (Kjeldsen et al. 1988; Itzkowitz et al. 1989). In addition, our previous reports demonstrated that altered expression of carbohydrate sequences was often associated with carcinogenesis of the uterine endometrium (Inoue et al. 1987, 1990a). The present study was designed to investigate the distribution of STn in benign, hyperplasia and adenocarcinoma of the endometrium, and to determine the potential utility of monoclonal antibody (TKH-2) specific for STn in practical surgical pathology.

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Materials and methods

Tissue blocks from 64 patients with endometrial abnormalities were collected from the pathology files of the Department of Obstetrics and Gynaecology at Osaka University Medical School, the Department of Pathology at Koseinenkin Hospital, the Department of Pathology at Keisatsu Hospital and the Department of Pathology at Sumitomo Hospital. They consisted of 5 cystic hyperplasias, 4 adenomatous hyperplasias, 12 atypical hyperplasias and 43 endometrial cancers (34 well, 6 moderately, and 3 poorly differentiated adenocarcinomas). Normal endometrial tissues were also obtained from the surgical specimens of 32 patients treated for uterine myoma or carcinoma in situ of the uterine cervix at the Department of Obstetrics and Gynaecology, Osaka University Medical School. Tissue samples fixed in 10% formalin and embedded in paraffin were cut into 4- to 5-μm-thick sections. They were then stained by routine histopathological techniques. The sections were independently examined by at least three investigators. Most disagreements centred around the differential diagnosis between atypical hyperplasia and well-differentiated adenocarcinoma. Such cases were re-examined with a double-headed microscope until a consensus was achieved according to the histological typing system of the World Health Organization (Poulsen et al. 1975).

The tissue localization of STn antigen was determined immunohistochemically with the avidin-biotin-peroxidase complex method for 10% formalin-fixed and paraffin-embedded sections (Hsu et al. 1981). Briefly, the tissue sections were deparaffinized, dehydrated in graded ethanol and immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity. The slides were then washed in 0.05 M phosphate-buffered saline (PBS), pH 7.4, and were subsequently treated with 10% horse serum to inhibit nonspecific binding of antisera. Primary monoclonal antibody (TKH 2) against STn antigen was applied for 1 h at room temperature. After rinsing with PBS, the sections were incubated for 30 min with biotin-labelled goat anti-mouse IgG (Vector Labs, Burlingame, Calif.). They were then treated by the avidin-biotin-peroxidase complex (Vector Labs) at room temperature. Sites of peroxidase activity were visualized with 0.1% 3,3-diaminobenzidine-tetrahydrochloride containing 0.02% hydrogen peroxide in PBS. The slides were lightly counterstained with haematoxylin. Negative controls included sections incubated with normal mouse serum in place of the primary specific antibody. Cellular localization of the antigenic sites was determined by two investigators using a double-headed light microscope. The relative number of immunoreactive cells was scored from 0 to 3 as follows: 0, negative; 1, less than 10%; 2, 10–50%; 3, more than 50%.

Results

The results of immunostaining for STn antigen expression in normal, hyperplastic and malignant tissues of the endometrium are summarized in Table 1. STn was not expressed in either glandular or stromal components of proliferative endometrium, while it was present in 13 of 18 secretory phase endometrial tissues. Positive staining was observed on the apical portion of glandular cells in 7 of 12 early secretory phase endometrium (Fig. 1A). Positive glands varied from a few to most in the functional layer of the endometrial tissues, while the basal endometria failed to demonstrate reactivity. All 6 late phase secretory endometria showed intense staining in both the cytoplasm of glandular cells and secreted products within most glandular lumina located in the functional layer (Fig. 1B). Less frequent and weak staining was seen in the luminal surface. There was no reactivity in the glands in the basal layer or atrophic endometrium.

Table 1. Immunodetection of sialyl-Tn antigen in uterine endometrial tissues

Diagnosis	No. of cases	Staining score[a]			
		0	1	2	3
Normal					
Proliferative phase	10	10	0	0	0
Secretory phase					
Early	12	5	3	3	1
Late	6	0	0	0	6
Postmenopausal	4	4	0	0	0
Hyperplasia					
Cystic	5	5	0	0	0
Adenomatous	4	4	0	0	0
Atypical	12	9	3	0	0
Adenocarcinoma					
Well differentiated	34	4	11	15	4
Moderately differentiated	6	2	1	3	0
Poorly differentiated	3	1	1	0	1

[a] The relative numbers of immunoreactive cells were scored from 0 to 3 as follows: 0, negative; 1, <10%; 2, 10%–50%; 3, >50%

No expression of STn was observed in cases of cystic and adenomatous hyperplasia. However, 3 cases of atypical hyperplasia showed faint STn expression, although the antigen was negative in 9 of 12 cases; STn was positive on the luminal surface of a few glands with severe morphological atypia showing questionable invasion of the surrounding stroma (Fig. 2A). STn antigen was also expressed in the atypical hyperplastic areas adjacent to a carcinoma in 4 of 5 cases observed (Fig. 2B), while it was negative in the normal-appearing epithelium of these cases.

Thirty-six (84%) of 43 adenocarcinomas expressed STn, with the percentage of positive tumour cells ranging from a few to 100%. The antigen was observed to be distributed mainly in the whole cytoplasm and occasionally in the apical portion. Intraluminal mucus secretions were also positive. Adjacent positive and negative cell aggregates coexisted in some cases, while in other areas positive cells were scattered among the negative cells (Fig. 3). The cellular distribution of STn did not correlate with the degree of histological differentiation or the extent of myometrial invasion.

Discussion

Carbohydrate chains on the cell surface are known to undergo great qualitative and quantitative changes in malignant transformation. These changes have been readily and widely detected in a number of human neoplasms using the recent hybridoma technique, which has made it possible to make monoclonal antibodies specific for the target antigens (Hakomori 1985). The clinical application of these monoclonal antibodies has mainly centred around the diagnostic use of tumour-associated antigen detection in the serum in monitoring cancer patients (Koprowski et al. 1981; Kannagi et al. 1986; Inoue et al. 1989b). Recently, some antibodies have been utilized as an immunocytochemical adjunct for the detection of cancer cells in cytological preparations of human

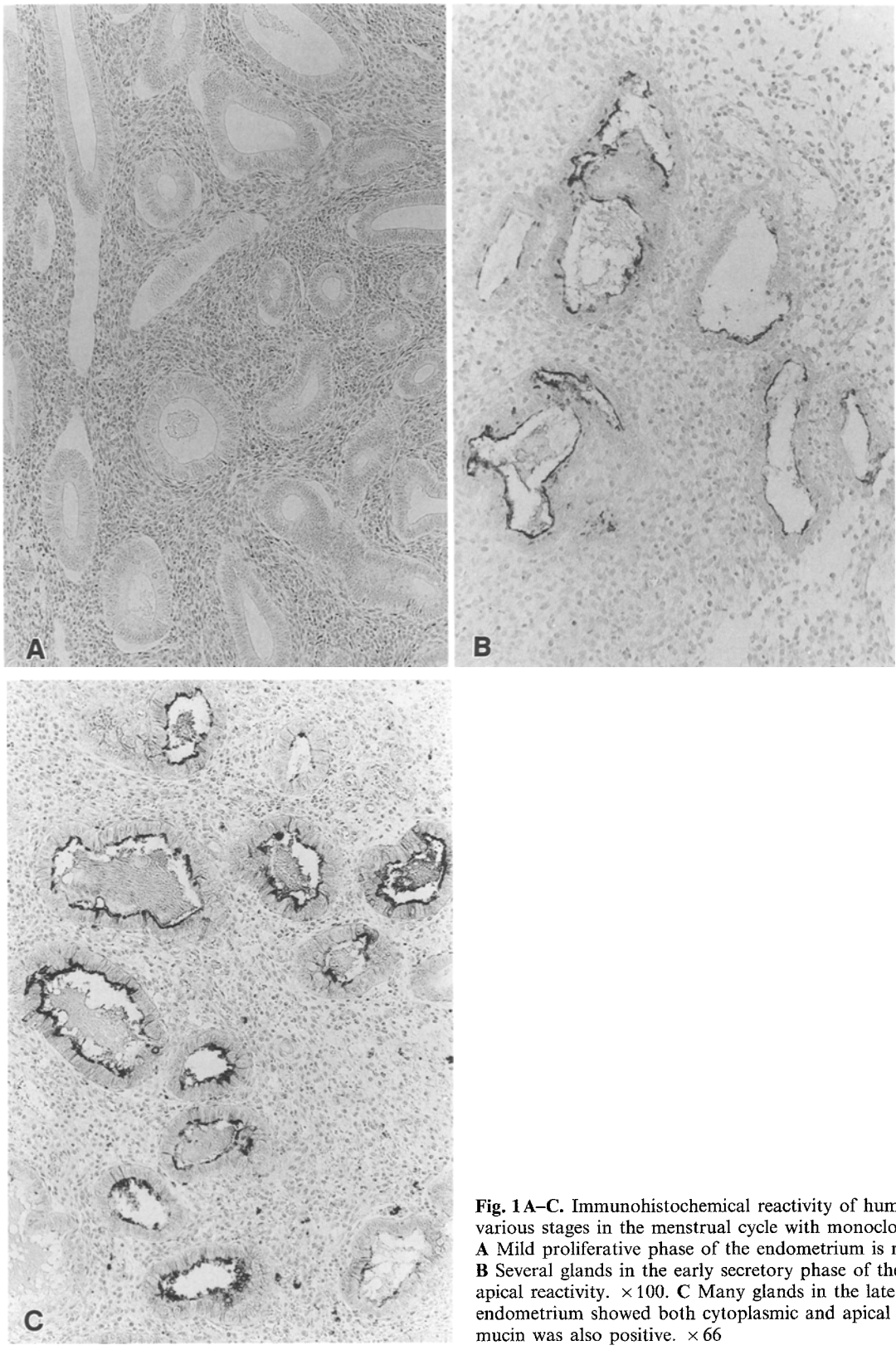

Fig. 1A–C. Immunohistochemical reactivity of human normal endometrium at various stages in the menstrual cycle with monoclonal antibody, TKH-2. **A** Mild proliferative phase of the endometrium is non-reactive. ×66. **B** Several glands in the early secretory phase of the endometrium showed apical reactivity. ×100. **C** Many glands in the late secretory phase of the endometrium showed both cytoplasmic and apical reactivity. Intraluminal mucin was also positive. ×66

effusions and biopsy specimens of tumour masses (Szpak et al. 1984; Martin et al. 1986; Johnston et al. 1986; Schlom 1986; Ranken et al. 1987). One such tumour-associated carbohydrated antigen, STn, seems to be an excellent candidate for such an approache in the field of gynaecological neoplasia, following the demonstration of its wide and intense reactivity with human colon cancers and its restricted reactivity with normal adult colonic tissues (Kjeldsen et al. 1988; Itzkowitz et al. 1989). In addition, frequent detection of STn in the se-

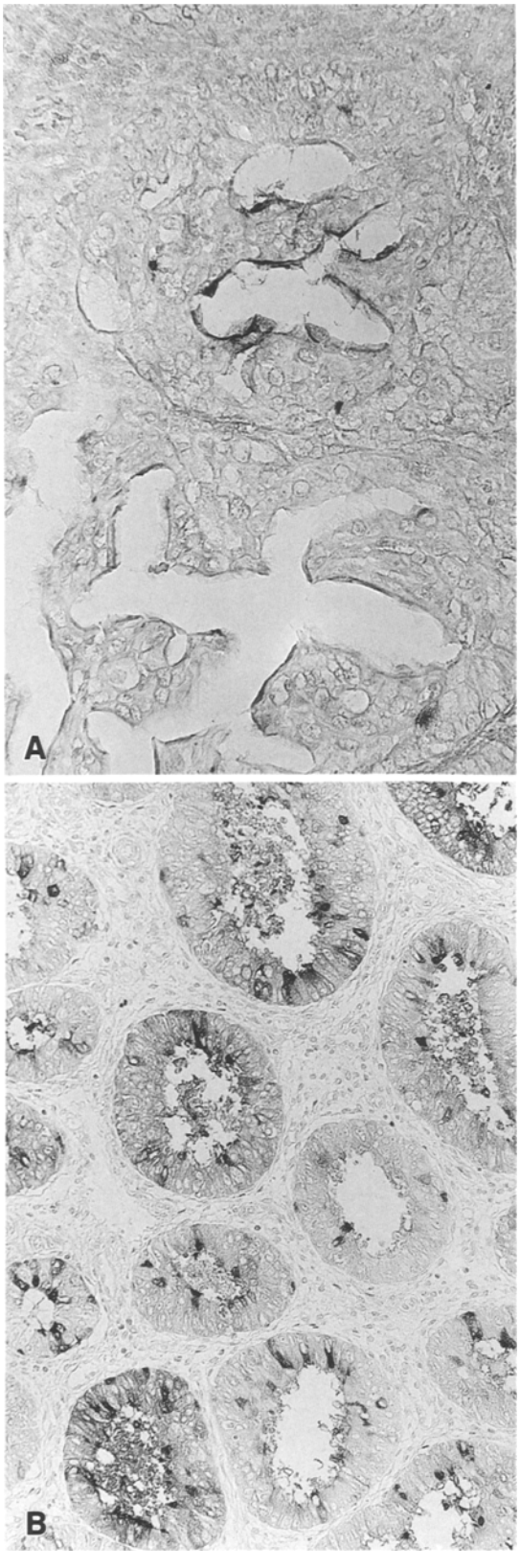

Fig. 2 A, B. Immunohistochemical reactivity of cystic, adenomatous and atypical hyperplasia with monoclonal antibody, TKH-2. **A** In the atypical hyperplasia, some areas showing the crowding glands, intraglandular bridges and stratified epithelium expressed sialyl-Tn antigen in the apical portion of the atypical cells. ×200. **B** Sialyl-Tn was expressed throughout cytoplasm of atypical cells adjacent to a carcinoma. ×100

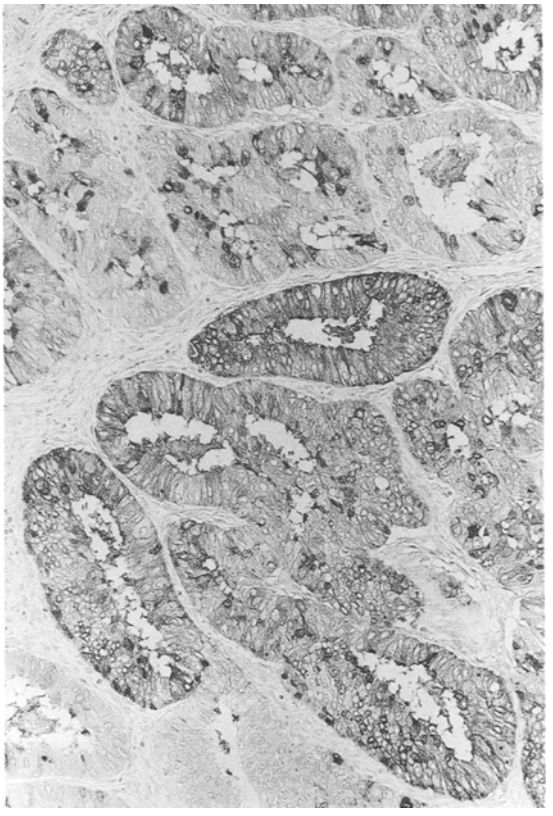

Fig. 3. Immunohistochemical reactivity of endometrial adenocarcinomas with monoclonal antibody, TKH-2. Sialyl-Tn antigen was distributed mainly in the cytoplasm of cancer cells and also in the apical cytoplasm and luminal contents of the glands. ×66

rum of gynaecological cancer patients has been reported (Inoue et al. 1990b).

In the present study, we utilized an immunohistochemical method using a monoclonal antibody, TKH-2, to determine the expression of STn in formalin-fixed, paraffin-embedded tissue sections in an attempt to define the potential of that monoclonal antibody in clinical applications. STn was expressed in two-thirds of early secretory phase and all of late secretory phase endometrium. Proliferative, basal and atrophic endometria were consistently negative. It is noteworthy that the staining pattern shifted from apical to a more diffuse cytoplasmic and luminal content associated with the progression of the day in the mentrual cycle. A similar phenomenon was observed with use of other antibodies, termed CA-1 (Ferguson 1984), B72.3 (Thor et al. 1987) and MSN-1 (Poropatich et al. 1990). However, some other antibodies, including CSLEX (Inoue et al. 1990a) and MCA-97 (Inoue et al. 1989a) have shown pronounced expression of their corresponding carbohydrate antigens in proliferative endometrium. The biological significance or mechanism of altered expression of antigens in the normal cyclic endometrium is unknown, but hormonal modulation might be involved, as described by some investigators (Thor et al. 1987; Poropatich et al. 1990).

In hyperplasia of the endometrium, no case of either cystic or adenomatous forms showed STn antigen expression. Most cases of atypical hyperplasia also failed

to reveal the antigen. However, it is very interesting that a few cases of atypical hyperplasia revealed STn antigen expression in the area where intraglandular bridges, crowding glands and severe cellular atypia were present. Our hypothesis, that STn expression is closely related to the early stage of transformation from hyperplasia to cancer is further supported by the following findings. Recent quantitative radiometric immunoassay of STn has shown higher STn antigen levels in cystic fluids of ovarian cancers, including low-potential malignant tumours compared with benign tumours (Inoue et al. 1990b), and STn antigen was also expressed in the atypical hyperplastic areas adjacent to a carcinoma in 4 of 5 cases observed, in contrast to the complete lack of reactivity in the normal-appearing epithelium within these cases.

Stn antigen was expressed in more than 80% of endometrial adenocarcinomas. The intensity and incidence of staining in cancers varied widely from case to case and from area to area within one case. In general, however, the staining was diffusely cytoplasmic, with strong staining associated with the luminal surface. Extracellular mucin was also positive. These staining patterns are quite similar to those for other mucin-type carbohydrate antigens, including blood type antigens (Inoue et al. 1987, 1990a), TAG-72 (Thor et al. 1987) and MNS-1 (Poropatich et al. 1990). The epitope of TAG-72, defined by monoclonal antibody B72.4, has recently been suggested to be identical to STn antigen by some investigators (Kjeldsen et al. 1988; Itzkowitz et al. 1989), whereas Springer et al. (1986) showed that B72.3 antibody agglutinates Tn erythrocytes and assumed it to be directed to Tn antigen. Thor and collaborators (1987) detected TAG-72 in 100% of endometrial adenocarcinomas and in a preliminarly report stated that it was also present in hyperplastic lesions and appeared to correlate with the severity of the histological abnormality. The incidence of staining we find in tumours is less frequent than in their report but the staining pattern is quite similar. The epitope of THK-2 may be slightly different from that of B72.4.

STn was extensively expressed in adenocarcinomas of the uterine endometrium but was not present in hyperplastic proliferative endometrium. This suggests that it has potential as a useful diagnostic aid for the pathologist who is forced to make the difficult differential diagnosis between adenocarcinoma and hyperplasia, (especially atypical hyperplasia) using small biopsies.

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