Immunohistochemical Localization of the Mouse Stage-specific Embryonic Antigen 1 in Human Tissues and Tumors

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INTRODUCTION

The SSEA-13 is a carbohydrate antigenic determinant defined by a monoclonal antibody produced by immunizing mice with murine embryonal carcinoma F9 cells (8, 9, 13). Although all mouse embryonal carcinoma cell lines react with this monoclonal antibody, we could not demonstrate SSEA-1 on any other mouse tumor cell lines. Although SSEA-1 is selectively expressed on mouse embryonic cells in a stage-specific manner, certain fetal and adult tissues also express this determinant (5–7). Nevertheless, despite widespread distribution of SSEA-1 in normal murine tissues, the monoclonal antibody to SSEA-1 injected i.v. into tumor-bearing mice can be localized to embryonal carcinoma (1).

In a previous study (2), we have shown that the monoclonal antibody to SSEA-1 reacts with human yolk sac carcinoma as well as germ cells in human fetal (but not adult) testis. Because of the potential diagnostic or therapeutic value of the anti-SSEA-1 antibody in clinical medicine, it was essential to determine whether this antibody reacts with other human tumors and normal human tissues. We report that antibody to SSEA-1 reacts with many human tissues and that tumors originating from immunoreactive tissues also express this antigenic determinant. Most tumors, however, show considerable heterogeneity and are composed of both SSEA-1-positive and -negative cells. We also show that, in the breast and ovary, SSEA-1-positive tumors develop from the epithelial tissues that are normally unreactive with this monoclonal antibody.

MATERIALS AND METHODS

Tissue Samples and Tumors. The normal tissues listed in Table 1 were removed within five hr of death from 2 male and 2 female autopsy cases at the Hahnemann Medical College, Philadelphia, Pa. All specimens were immediately frozen in 2-methylbutane, precooled in liquid nitrogen, and sectioned immediately on a cryostat or stored at −70°C. Fresh tumor samples were obtained from specimens surgically removed at Hahnemann and similarly processed. In addition, paraffin-embedded tumors from previous surgical cases were sectioned and tested for SSEA-1.

Antiserum to SSEA-1. The anti-SSEA-1 monoclonal antibody was produced previously (13) by fusing the spleen cells of a mouse immunized with the nullipotent mouse embryonal carcinoma cell line F9 with cells of the murine myeloma cell line P3X63Ag8. From this fusion, a cloned hybrid cell line was obtained which secreted monoclonal antibodies of the IgM isotype. This monoclonal antibody defined the SSEA-1 antigenic determinant. Ascites fluid from mice injected i.p. with this antibody-secreting hybrid cell clone was used throughout this investigation. It was diluted 1:10 in PBS containing bovine serum albumin (1 mg/ml) and sodium azide (0.04 mg/ml). Ascites fluid (diluted 1:10 above) from a mouse injected i.p. with the P3X63Ag8 myeloma line was used as the primary antibody on control sections.

Immunohistology. Indirect immunofluorescence or immunoperoxidase tests were performed on cryostat sections which were briefly fixed in cold acetone (4°C) for 10 min. Paraffin sections were first deparaffinized in xylene, cleared and rehydrated in graded alcohols, treated with trypsin (1:250) (M. A. Bioproducts, Walkersville, Md.) for 30 min at 37°C, and rinsed 3 times in PBS. The slides were incubated with either anti-SSEA-1 or control ascites for 1.5 hr at room temperature in a humidified chamber. After rinsing in PBS (4°C), either horseradish peroxidase-conjugated or fluorescein-conjugated goat (IgG fraction) anti-mouse IgM (heavy chain specific) (Cappel Laboratories, Cochranville, Pa.) were similarly incubated on the slides for 1 hr. After a rinsing in PBS, the slides were either reacted with diamino benzidine (5) for light microscopy or mounted with glycerine for fluorescent microscopy.

RESULTS

SSEA-1 in Normal Tissues

The normal tissues tested for SSEA-1 are listed in Table 1. In each tissue where SSEA-1 was detected, reactivity was always limited to the epithelial components. With the exception of the central nervous system (see below), no reactivity was detected on the stromal or connective tissue elements in any tissue.

Hemopoietic-Lymphoid Tissues. In peripheral blood smears

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2 To whom requests for reprints should be addressed.

3 Abbreviations used are: SSEA-1, stage-specific embryonic antigen 1; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocytes.

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Expression of SSEA-1 in normal human tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Immunohistochemical reactivity for SSEA-1</th>
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<tr>
<td>Hemopoietic/lymphoid organs</td>
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<tr>
<td>RBC</td>
<td>–</td>
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<tr>
<td>Polymorphonuclear leukocytes</td>
<td>++</td>
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<tr>
<td>Lymphocytes</td>
<td>–</td>
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<tr>
<td>Bone marrow (presumptive precursors of PMN)</td>
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<tr>
<td>Thymus</td>
<td>–</td>
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<tr>
<td>Tissue macrophages including Kupffer cells</td>
<td>++</td>
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<tr>
<td>Spleen</td>
<td>++</td>
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<tr>
<td>Nervous system</td>
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<tr>
<td>Cerebrum</td>
<td>++</td>
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<tr>
<td>Cerebellum</td>
<td>+/−</td>
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<tr>
<td>Pons</td>
<td>++</td>
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<tr>
<td>Spinal cord</td>
<td>++</td>
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<tr>
<td>Peripheral nerves</td>
<td>–</td>
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<tr>
<td>Digestive tract</td>
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<tr>
<td>Esophagus</td>
<td>–</td>
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<tr>
<td>Stomach</td>
<td>+/−</td>
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<tr>
<td>Small intestine</td>
<td>+/−</td>
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<tr>
<td>Colon</td>
<td>+/−</td>
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<tr>
<td>Liver</td>
<td>–</td>
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<tr>
<td>Salivary glands</td>
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<tr>
<td>Acini</td>
<td>–</td>
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<tr>
<td>Ducts</td>
<td>+/−</td>
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<tr>
<td>Urinary tract</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Pelvis</td>
<td>+/−</td>
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<td>Ureter</td>
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<tr>
<td>Cardiovascular system</td>
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<tr>
<td>Heart</td>
<td>–</td>
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<tr>
<td>Arteries</td>
<td>–</td>
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<td>Veins</td>
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<td>Skeletomuscular system</td>
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<tr>
<td>Skeletal muscle</td>
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<td>Skin</td>
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<td>Connective tissue</td>
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<td>Epidermis</td>
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<tr>
<td>Skin appendages</td>
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<td>Reproductive system</td>
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<tr>
<td>Testis</td>
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<td>Ovary</td>
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<tr>
<td>Epididymis</td>
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<td>Fallopian tube</td>
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<td>Uterus</td>
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<td>Cervix</td>
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<td>Vagina</td>
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<td>Mammary gland</td>
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<td>Ducts</td>
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<tr>
<td>Pregnancy alveoli</td>
<td>+/−</td>
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<tr>
<td>Respiratory tract</td>
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<tr>
<td>Trachea</td>
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<td>Bronchi</td>
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<td>Lung</td>
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<td>Endocrine glands</td>
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<td>Pancreas</td>
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<td>Thyroid</td>
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<td>Parathyroid</td>
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<tr>
<td>Adrenal glands</td>
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<tr>
<td>Cortex</td>
<td>–</td>
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<tr>
<td>Medulla</td>
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* Undetectable, –; strong, +++; weak, +/−; moderate, +.

and bone marrow smears, SSEA-1 was detected on PMN and their presumptive immature precursors (Fig. 1A). Many cells in these smears did not react but were not further characterized. PMNs, as well as tissue histiocytes which also reacted strongly with the antibody, were commonly seen in tissue sections (Figs. 4 and 6A).

In the spleen, thymus, and lymph nodes, the only cells reactive for SSEA-1 were PMNs and histiocytes (Fig. 1B).

Nervous System. SSEA-1 was detected only in the central nervous system, i.e., the brain and spinal cord. No reactivity was seen in the peripheral nerves. In the brain, the cerebrum, cerebellum, and pons all reacted strongly. SSEA-1 was detected on both neuronal cells and supportive glial cells in both the white and gray matter of the brain and spinal cord (Fig. 2). SSEA-1 was seen in the cytoplasm and on the cell membrane of cells in the gray matter but appeared bound to the cell surface in the white matter (Fig. 2).

Digestive Tract. In the salivary glands, SSEA-1 was detected on the cell surface and in the cytoplasm of ductal cells but was not present on serous or mucinous acinar cells (Fig. 3A).

SSEA-1 was not detected in the esophagus, while in the stomach strong cytoplasmic and cell surface reactivity for SSEA-1 was seen on cells in the crypts of the mucosa (Fig. 3B). Reactivity on the epithelium of the villi was less obvious, particularly near the lumenal surface (Fig. 3C). In the small intestine, only weak reactivity was seen and was limited to the cells of the crypts. In the colon, strong to moderate cell surface and cytoplasmic reactivity was seen on all epithelial cells of the mucosa but appeared strongest on cells in the crypts (Fig. 3D).

No reactivity was detected in the pancreas. In the liver, only Kupffer cells were positive for SSEA-1.

Urinary Tract. In the kidney, cytoplasm and cell surface reactivity for SSEA-1 was clearly detected in most tubules in the cortex and on leukocytes (Fig. 4). All medullary tubules were negative as were some tubules in the cortex, the glomeruli, and the interstitium. Weak to moderate reactivity was seen on the epithelium of the pelvis, ureter, and bladder (not shown).

Reproductive Tract. SSEA-1 was not found in the testis or ovary, although it was clearly detected on the cell surfaces of epithelial cells lining the ductus epididymis and endocervix (Fig. 5). The epithelium of the fallopian tube and endometrium was weakly reactive with the antibody.

Mammary Gland. Ductal cells of the resting mammary gland were unreactive with the antibody (Fig. 6A). In the mammary gland at 8 months of pregnancy, alveoli were abundant, and both the cytoplasm and the cell surface of the alveoli strongly expressed SSEA-1 (Fig. 6B).

Endocrine Glands. A diffuse reaction with the antibody was seen in the adrenal medulla, while the adrenal cortex was negative. In the pituitary, SSEA-1 was detected in both the neuro- and adenohypophysis. Both cytoplasmically stained cells and negative cells were seen in the adenohypophysis (Fig. 7A), while a diffuse reaction was seen in the neurohypophysis (Fig. 7B).

As shown in Table 1, all other endocrine glands tested including the pancreatic islets, thyroid, and parathyroid did not express SSEA-1.

Respiratory Tract. No reactivity for SSEA-1 was detected on the trachea, bronchi, and parenchymal cells of the lung. However, reactivity was quite apparent on alveolar macrophages and histiocytes.

Skin, Cardiovascular, and Skeletomuscular Systems. No reactivity was detected on cells of the epidermis, skeletal muscle, heart, or great vessels. Dermal glands were reactive (not shown).
SSEA-1 in Human Tissues and Tumors

Fig. 1. Reaction of antibody to SSEA-1 as detected by immunofluorescence on a bone marrow smear (A). Reaction product is seen in the cytoplasm of all cells forming this nest of PMN precursors. x 400. B, immunoperoxidase on a spleen section. x 250.

Fig. 2. Immunofluorescent localization of SSEA-1 on a section of cerebrum. Reaction is seen on both white (W) and gray (G) matter. x 250.

SSEA-1 in Tumors

The tumors tested for SSEA-1 are listed in Table 2. Germ cell tumors have been analyzed previously (2) and are not included in this study. As indicated in Table 2, SSEA-1 was not detected in connective tissue tumors such as fibrosarcoma and leiomyoma but could be detected in many carcinomas. With only 2 exceptions (breast and ovary), expression of SSEA-1 was limited to tumors arising from SSEA-1-positive normal tissues. However, the extent of SSEA-1 expression in such tumors varied. Myelogenous leukemia cells reacted strongly with the antibody while 2 lymphocytic leukemias were unreactive. In each of the adenocarcinomas of the colon and stomach and in a transitional cell carcinoma of the kidney, nearly as many tumor cells were negative for SSEA-1 as were positive (Fig. 8). Positive tumor cells showed both cytoplasmic and cell surface staining (Fig. 8). Both poorly differentiated and well-differentiated tumors were SSEA-1 positive or negative.

While the normal ductal epithelium of the mammary gland was unreactive for SSEA-1 (Fig. 6A), all ductal cell carcinomas of the mammary gland tested had moderate to strong cytoplasmic reactivity (Fig. 9). In contrast to gastrointestinal tumors, few SSEA-1-negative tumor cells were seen in these mammary carcinomas. With the exception of a single ovarian tumor (Table 2), all other tumors arising in SSEA-1-negative normal tissues were likewise unreactive for SSEA-1.

Paraffin-embedded tissues containing positive cells reacted essentially in the same manner as the freshly frozen material. However, the intensity of the reaction and the number of reactive cells were always lower than in freshly frozen tissues of the same organ or tumor.
DISCUSSION

The antigenic determinant recognized by the monoclonal antibody to SSEA-1 was originally found on mouse embryonal carcinoma cells, and then later it was discovered to be expressed on defined populations of embryonic, fetal, and adult mouse cells (5–7), as well as on tissues of other species (2, 11). The present study shows that the Lc haptenic determinant (8, 9) recognized by this monoclonal antibody is widely distributed in normal human tissues. We have previously reported that granulocytes from peripheral blood express SSEA-1 (11), and we now report the expression of this antigenic determinant on fixed tissue macrophages. As in the mouse, SSEA-1 was found on portions of the human urogenital tract, gastrointestinal tract, and central nervous system. However, even in these anatomic locations, its expression in the 2 species differed. For example, SSEA-1 was localized to the cortical tubules, medullary tubules, and pelvic epithelium in the mouse kidney (7), whereas in the human kidney SSEA-1 was detected only in the cortex and on the pelvic epithelium. Thus, although obviously a heterogenetic antigen like the best known heterogenetic antigen, the Forssmann antigen, SSEA-1 is not specific for one cell type or tissue but appears on different cells in different species. Since the SSEA-1 antigenic determinant, like the blood group antigens, could appear on glycolipid, glycoprotein, or oligosaccharide molecules and since we have not determined in which form it appears in any organ in these species, the anatomic disparity could be even more complex if one were to take into account the potential biochemical variability. However, if the tissue localization mirrors the activity of a glycosyltransferase, these enzymes appear to fucosylate the proper donor molecule, be it attached to a lipid or protein moiety. Also, the limited number of cases examined in this study may not reflect all possible patterns of expression which might be affected by individual genetic differences such as blood group type or secretor status. Despite the widespread distribution of SSEA-1 in human tissues, not all cells in the SSEA-1-positive tissues were immunoreactive with the anti-SSEA-1 antibody. Thus, for example, SSEA-1 could be detected in the striated ducts but not in the acinar cells of the salivary gland, in the endometrial glands but not in the stroma, and in the adrenal medulla but not in the cortex. Hence, due to its anatomically specific distribution in various tissues, SSEA-1 could be used as a tissue-specific cell marker.

SSEA-1 could also be used as a marker for the functional state of certain cells, most notably in the breast, where its expression appears to be under the influence of hormonal stimuli. SSEA-1 was not expressed in the normal breast epithelium, but it appeared in the acini of the lactating breast. In this respect, SSEA-1 has some resemblance to the antigens recognized by the monoclonal antibodies raised to human milk and reactive with lactating but not resting human breast tissue (3, 4). It is of interest to note that the antibodies described by Foster et al. (4), although produced in response to immunization with human milk, reacted with numerous other human tissues. Springer et al. (14) have also described the expression of blood group T-determinant on cells of breast carcinomas.

Anti-SSEA-1 antibodies reacted not only with normal human tissues but with numerous human tumors as well. Cell lines derived from human myeloid leukemia, colorectal carcinomas, choriocarcinomas, and differentiated hepatomas have been previously shown to be SSEA-1 positive (11). Extension of this study to tumors in situ allows us to generalize. SSEA-1 was detected on most adenocarcinomas tested, irrespective of their site of origin. Mesenchymal tumors, benign and malignant, were unreactive. Tumors that were immunoreactive originated not only from SSEA-1-reactive epithelia like colon or stomach but also from SSEA-1-negative tissues like breast and ovary. The tumors that were positive were, however, composed of both immunoreactive and nonreactive cells. This indicates that the tissue-specific antigenic determinants may be preserved or lost during neoplastic transformation and that antibody to SSEA-1 reveals a considerable heterogeneity within tumor cell populations.

Like numerous other tumor markers detected by polyclonal (10) or monoclonal antibodies (3, 12), SSEA-1 is not restricted to neoplastic cells. As with other tumor markers, the SSEA-1 determinant has no known function. However, in contrast to many tumor antigens defined by monoclonal antibodies, SSEA-1 has been biochemically characterized, and its distribution on normal tissues has been extensively studied in both man and mouse.

Understanding of the biochemical nature of the antigenic determinant coupled with the knowledge about its anatomic distribution could be used for further studies to elucidate the regulatory mechanisms governing its expression on malignant cells and also possibly its role in the cell-to-cell interactions. As a prototype of an oligosaccharide antigen linked to glycolipids or glycopeptides, SSEA-1 could serve as an excellent marker to obtain more information about the changes of cell surface carbohydrates in cancer.

REFERENCES

Fig. 3. Reaction of antibody to SSEA-1 as detected by the following. A, immunoperoxidase on a section of salivary gland. D, ductal cells; A, acini. × 250. B, immunofluorescence on a section through the crypts of the stomach. × 250. C, immunofluorescence on a section through the upper neck of the gastric glands. × 250. D, immunoperoxidase on a section through the crypts of the colon. × 250.
Fig. 4. Immunoperoxidase localization of SSEA-1 on a section of the cortex of a kidney; G, glomeruli; arrows, leukocytes; X, negative tubules. × 100.

Fig. 5. Immunoperoxidase localization of SSEA-1 on sections through: A, epididymis; B, endocervix. × 250.
Fig. 6. Immunoperoxidase localization of SSEA-1 on sections through: A, mammary gland duct (arrows, leukocytes); B, acini of the mammary gland in pregnancy. x 250.

Fig. 7. Immunofluorescent localization of SSEA-1 on sections of the pituitary. A, adenohypophysis; B, neurohypophysis. x 250.
Fig. 8. Immunofluorescent localization of SSEA-1 in tumors. A, adenocarcinoma of the colon (both positive and negative (N) well-differentiated glands are present). × 250. B, adenocarcinoma of the stomach composed of positive and negative glands. × 100. C, transitional cell carcinoma of the kidney. The group of tumor cells consists of centrally located positive and peripherally located negative cells. × 250.
Fig. 9. Immunoperoxidase localization of SSEA-1 in ductal cell carcinomas of the mammary gland. A, solid foci of carcinoma. × 100. B, a focus of tumor cells at higher magnification to demonstrate membranous location of the reaction product. × 250. C, infiltrating ductal cell carcinoma. × 100.
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