Changes in Lectin Binding Patterns of Mouse Male Germ Cells (Gonocytes) during Prespermatogenesis

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(Received 26 October 1998/Accepted 19 December 1998)

ABSTRACT. The distribution of sugar residues in gonocytes of the differentiating mouse testis was examined by light microscopy using 22 different kinds of lectins. Characteristic binding patterns of sWGA, VVA, and LEA in gonocytes were observed during prespermatogenesis. sWGA preferentially bound to the cytoplasm and plasma membrane of gonocytes on 16.5 days post coitus (dpc). Its reaction decreased thereafter and almost disappeared on 1.5 days post partum (dpp), but reaction reappeared on 4.5 dpp and continued until 6.5 dpp. The VVA reaction was recognized in a few gonocytes on 0.5 dpp, and remained strong until 6.5 dpp. LEA reacted strongly in the plasma membrane and cytoplasm of gonocytes from 0.5 dpp to 6.5 dpp. The present study indicates that sWGA, VVA, and LEA are useful markers for gonocytes, and the appearance or disappearance of sWGA and VVA may be related to the differentiation of gonocytes during prespermatogenesis.—KEY WORDS: differentiation, glycoconjugate, gonocyte, lectin, prespermatogenesis

J. Vet. Med Sci. 61(5): 465–470, 1999

In an early postnatal life, the cells of the seminiferous epithelium undergo developmental changes that are ultimately critical for establishment of spermatogenesis. In particular, the gonocytes (precursors of germ cell lineage) in the seminiferous tubule resume their mitotic activity, relocate on the basement membrane, and give rise to spermatogonia. At first, they proliferate until about 14.5 days post coitus (dpc) to 16.5 dpc [18, 27, 29], and then are arrested in the G1 phase of the cell cycle. After birth, the gonocytes reinitiate to divide [13, 21]. They extend cytoplasmic processes to the basement membrane and move from the central region toward the base [8, 25]. Thus, spermatogenic cells are placed in an environment likely to be crucial for their further maturation [7, 17, 20, 24]. These stages are called prespermatogenesis [10, 30]. Recent investigations indicate that the change in carbohydrate distribution in the cytoplasm and plasma membrane of various cells is strongly related to cellular differentiation and to cell-to-cell interactions [9, 23]. Lectins, which bind to specific sugar residues, have been used as potent probes for identification of the carbohydrate molecules on cell surface and in cytoplasmic organelles. Important roles of the complex carbohydrates on the cell surface have been shown by lectin histochemistry [5, 12, 22, 26]. In developing gonads, stage-specific changes in carbohydrates on the surface of germ cells have been reported using lectins [2–4, 14] or monoclonal antibodies such as SSEA-1, EMA-1, EMA-6, Le^x, EE2 and 4C9 [6, 11, 16, 19, 31] as probes. So far, however, little information has been available on the lectin binding pattern during prespermatogenesis. In the present study, the changes in the distribution of surface and cytoplasmic glycoconjugates in mouse gonocytes were examined by light microscopy using lectins as probes.

MATERIALS AND METHODS

Preparation of animals: Virgin female 8- to 12-week-old mice from the inbred strain C57BL/6J were mated with males of the same strain. All animals were maintained in our laboratory. They were housed on a standard program of 14 hr light: 10 hr dark and were given food and water *ad libitum*. Females were placed with males overnight and the presence or absence of spermatozoa in the vaginal smear was examined in the next morning. The day when spermatozoa were found was designated as 0.5 dpc of gestation. Testes were obtained from fetuses 16.5 dpc to 18.5 dpc and new borns 0.5 day post partum (dpp) to 6.5 dpp.

Staining with lectins for light microscopy: The lectin probes used in this study were Concanavalin A agglutinin (ConA, *Canavalia ensiformis)*, *Bauhinia purpurea* agglutinin (BPA), succinylated wheat germ agglutinin (sWGA), wheat germ agglutinin (WGA, *Triticum vulgaris*), *Griffonia simplicifolia* agglutinin II (GS-II), *Datura stramonium* agglutinin (DSA), peanut agglutinin (PNA, *Arachis hypogea*), *Griffonia simplicifolia* agglutinin I (GS-I), soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), *Ulex europeus* agglutinin (UEA-I), *Bandeiraea simplicifolia* agglutinin I (BSA-I), *Pisum sativum* agglutinin (PSA), *Lens culinaris* agglutinin (LCA), *Phaseolus vulgaris Erythro* agglutinin (PHA-E), *Phaseolus vulgaris Leuco* agglutinin (PHA-L), *Sophora japonica* agglutinin (SJA), *Bandeiraea simplicifolia* agglutinin II (BSA-II), *Erythrina cristagalli* agglutinin (ECA), *Lycopersicon esculentum* (tomato) agglutinin (LEA), *Vicia villosa* agglutinin (VVA), and Jacalin. Biotinyl lectins (Vector Laboratories, Burlingame, CA, U.S.A.) were used along with avidin-biotinyl peroxidase complexes (ABC, Vector Lab.). Testes

were fixed in 10% formalin containing 2% calcium acetate for 2 days at 4°C. They were then dehydrated in a graded series of ethanol, infiltrated in xylene and embedded in paraffin. Deparaffinized sections (4 μm thick) were rehydrated and treated with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS), pH 7.2. The sections were incubated with biotinyl lectins (25 μg/m*l*) in 0.1% BSA-PBS for 60 min, and then washed with PBS. They were incubated with ABC for 30 min. After a rinse with PBS again, they were immersed in 3,3'-diaminobenzidine (DAB, 0.2 mg/m*l*)-H_2O_2 (0.005%) for 10 min, rinsed with distilled water, dehydrated, mounted and observed by light microscopy.

Control experiments: As control experiments to clarify binding specificity, deparaffinized sections were preincubated with appropriate hapten sugars for each lectin and then incubated in lectin solution containing hapten sugars. Non-specific staining was also checked by incubation with ABC and DAB-H_2O_2 solution.

RESULTS

As shown in Table 1, specific binding patterns were observed in three lectins (sWGA, VVA, and LEA). The detailed binding pattern of each lectin is described below. On 16.5 dpc, sWGA showed a granule-like reaction in the cytoplasm of gonocytes. The plasma membrane of gonocytes was also positive (Fig. 1A). The sWGA reaction decreased on 0.5 dpp (Fig. 1B), and almost disappeared on 1.5 dpp. However, the reaction reappeared in the cytoplasm and plasma membrane of gonocytes on 4.5 dpp and continued until 6.5 dpp (Fig. 1C). VVA reacted in only a part of interstitium on 16.5 dpc (Fig. 2A). The VVA reaction was observed in the cytoplasm (granule-like) and plasma membrane of gonocytes on 0.5 dpp (Fig. 2B) strongly positive in the cytoplasm of gonocytes on 4.5 dpp (Fig. 2C). The reaction remained to be positive until 6.5 dpp. LEA reacted strongly with the plasma membrane and cytoplasm of gonocytes on 16.5 dpc (Fig. 3A, B). The LEA-positive reaction continued until 6.5 dpp (Fig. 3C). A weak reaction of sWGA and LEA was occasionally observed in the plasma membrane of Sertoli cells during prespermatogenesis. BSA-I, PHA-L, PHA-E, DSA, ConA, WGA, ECA, PSA, and LCA showed a positive reaction in the plasma membrane and cytoplasm of gonocytes, Sertoli cells and other somatic cells. DBA, BPA, SBA, GS-I, GS-II, Jacalin, PNA, SJA, UEA-I, and BSA-II showed no definite reaction throughout prespermatogenesis. sWGA, VVA, and LEA- binding were completely inhibited by incubation in the presence of GalNac and GlcNac (Figs. 1D, 2D, and 3D) or by treatment with α-amylase (1 mg/m*l*, 37°C, 1 hr ;Wako, Osaka, Japan).

DISCUSSION

In the present study, changes in lectin binding patterns in mouse gonocytes during prespermatogenesis were examined.

Table 1. Changes in reactivity of lectin bindings to gonocytes during prespermatogenesis

	Fetal stage (dpc)			Postnatal stage (dpp)						
	16.5	17.5	18.5	0.5	1.5	2.5	3.5	4.5	5.5	6.5
sWGA	++	++	+	–~±	–	–	–	+	+	+
VVA	–	–	–	–~±	+	+	+	+	+	+
LEA	++	++	++	++	++	++	++	++	++	++

– (negative) ~ + (positive).

Some lectins bound to gonocytes (Table 1). The binding of the lectins examined in this study was classified into five different patterns. (1) sWGA reaction in gonocytes appeared from 16.5 dpc to 0.5 dpp, once disappeared on 1.5 dpp, and then reappeared on 4.5 dpp and continued until 6.5 dpp. (2) VVA reaction appeared in gonocytes on 0.5 dpp and continued until 6.5 dpp. (3) LEA reacted strongly in gonocytes throughout the stages examined. (4) BSA-I, PHA-L, PHA-E, DSA, ConA, WGA, ECA, PSA, and LCA reacted in most of the gonocytes and Sertoli cells. (5) DBA, BPA, SBA, GS-I, GS-II, Jacalin, PNA, SJA, and BSA-II showed no definite reaction throughout the stages examined. Because sWGA and LEA have specific affinity to β (1, 4) GlcNAc, and VVA has specific affinity to GalNAc$_1$α$_3$Gal, certain glycoconjugates containing β (1,4) GlcNAc and GalNAc$_1$α$_3$Gal residues are expressed in the cytoplasm and plasma membrane of gonocytes during prespermatogenesis. The changes in sWGA-and VVA-binding may especially relate to differentiation of gonocytes because the gonocytes begin to reproliferate from 1.5 dpp and differentiate into type A spermatogonia on 4.5 dpp [27]. In addition, these three lectins should be a selective marker for gonocytes. According to reports using the monoclonal antiserum against Forssman antigenic determinant (FA), monoclonal antigen 4B6, 3E10, these antigens appeared in most of mouse germ cells in fetal stages and disappeared after birth [15, 28]. As is well-known, although primordial germ cells of mouse embryos exhibit conspicuous alkaline phosphatase (ALP ase) activity [1], the reaction with ALPase in germ cells completely disappears after birth. The changes in ALPase activity during gonadal development are similar to those in binding patterns of some lectins (GS-I and BPA) [14]. Recently, EE2 antigen was found to localize on the cell surface of gonocytes from spermatogonia to preleptotene spermatocytes [19]. The antigenic molecule recognized by mAb EE2 is a novel glycoprotein with a molecular weight of 114 kDa. Due to its affinity with ConA and WGA lectins, and susceptibility to N-glycanase, it is suggested that the glycoprotein contains asparagine-linked sugar chains. The functional significance of the epitopes recognized by these markers is unclear. However, it is interesting that the appearance of the determinant detected by them during germ cell development correlates with the period when gonocytes become arrested in their mitotic activity. In addition, the determinant disappears a few days before re-entry of the gonocytes onto mitosis. Hence, it is possible that the

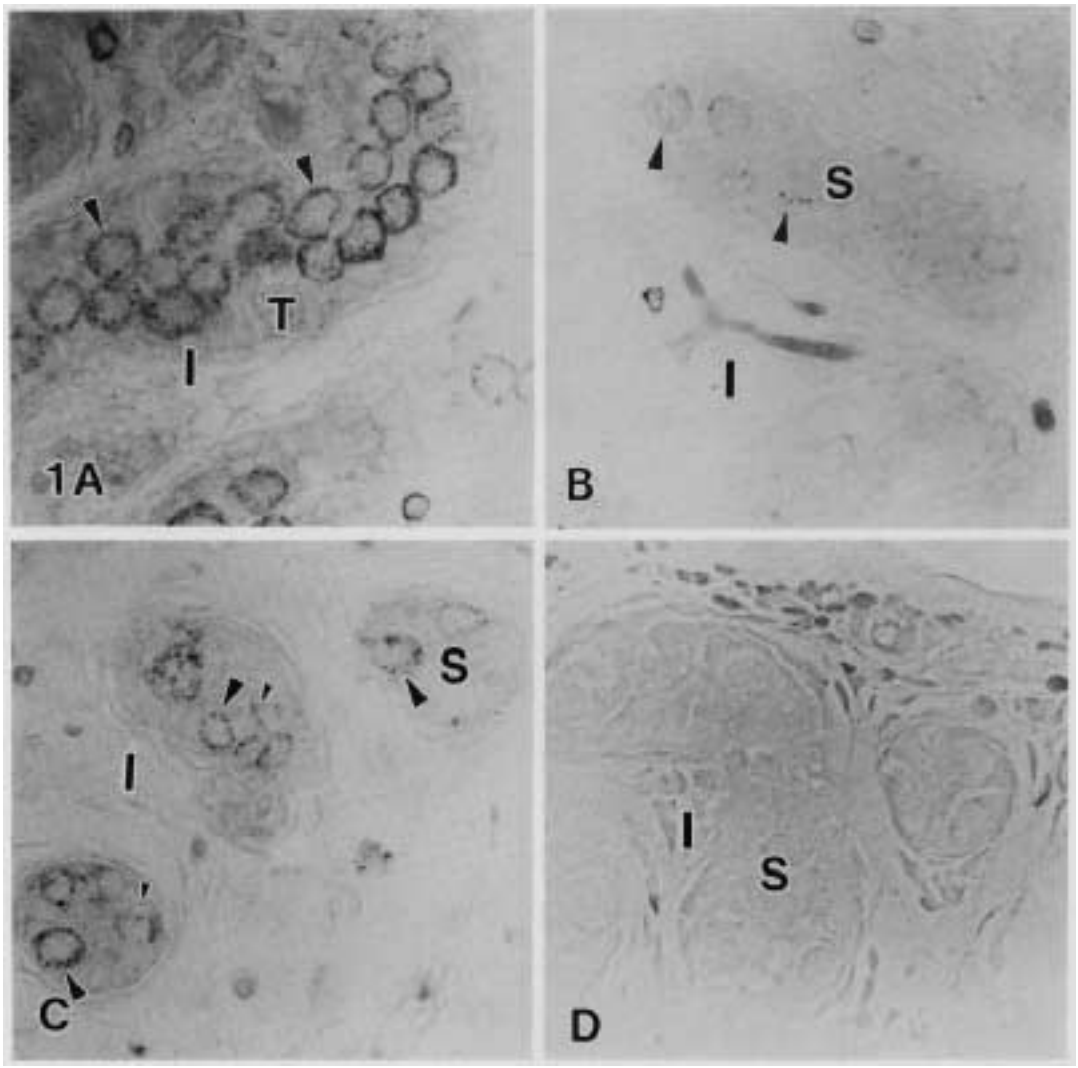

Fig. 1 A-D. Light micrographs showing sWGA bindings in the testis from 16.5 dpc to 4.5 dpp. Arrowheads: gonocytes, small arrowheads: Sertoli cells, I: interstitium, T: testicular cord, S:seminiferous tubule. A: 16.5 dpc. sWGA reacts in the cytoplasm, and plasma membrane of gonocytes B: 0.5 dpp. sWGA reaction decreases. C: 4.5 dpp. sWGA is detected in cytoplasm and plasma membrane of gonocyte. D: Control section incubated with biotinyl sWGA in the presence of 0.2 M N-acetylglucosamine. Positive reaction is completely inhibited. × 480 each.

appearance or disappearance of this determinant in gonocytes may be related to the differentiating process before the onset of spermatogenesis. Binding of FITC-lectins to gonocytes never changes after birth in the CD-1 strain mouse [2]. It is probable that lectin binding to germ cells may be different among strains [12]. In summary, sWGA, LEA and VVA are useful tools for studying the cell surface and cytoplasm carbohydrates of gonocytes during prespermatogenesis. The present findings indicate that there are changes in the glycosylation process of the VVA and sWGA-binding glycoconjugates during germ cell differentiation.

ACKNOWLEDGMENTS. The authors wish to thank Mr. Tsugiyama, I. (Department of Veterinary Anatomy, the University of Tokyo) for his expert care in keeping the laboratory animals. This work was supported in part by a grant-in-aid for Scientific Research Fund from the Ministry of Education, Science, Sports and Culture, Japan.

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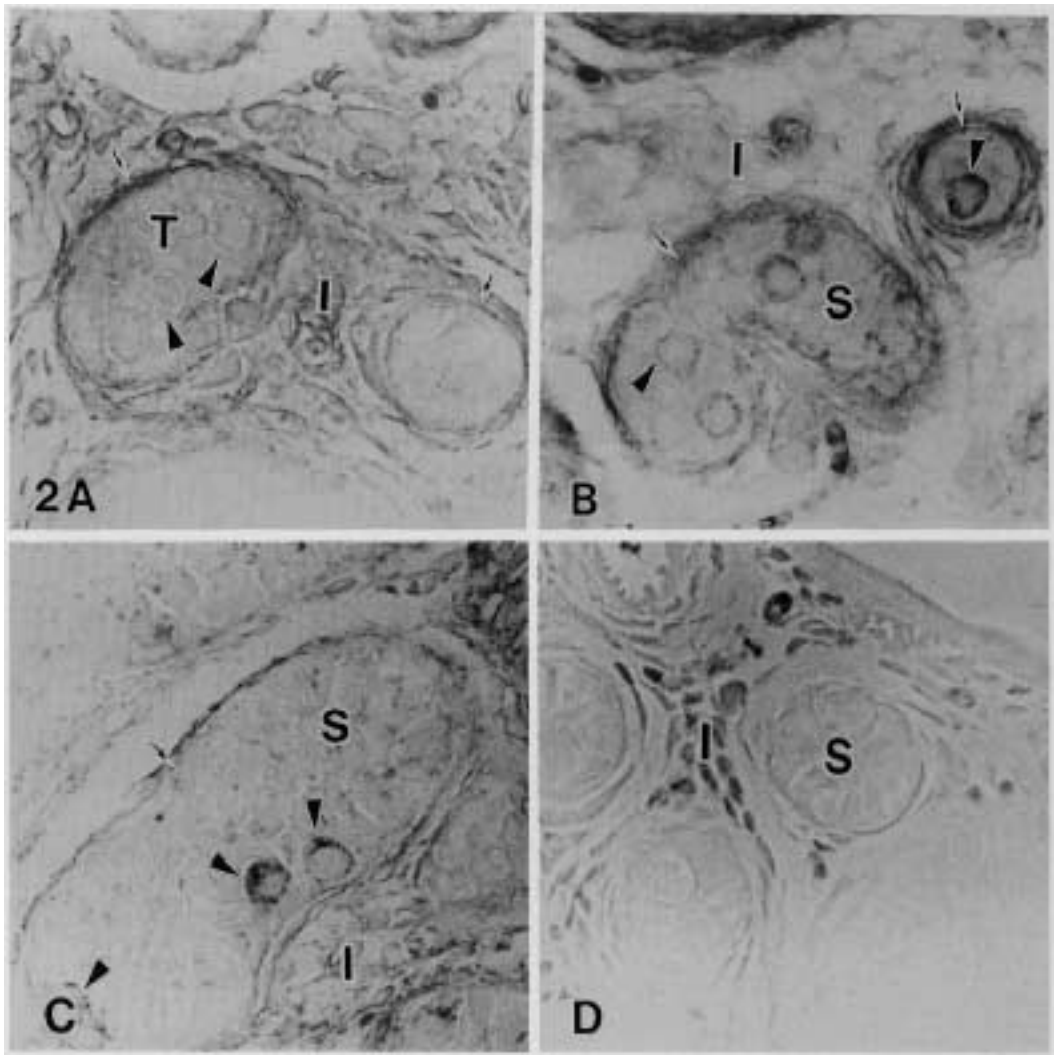

Fig. 2 A- D. Light micrographs showing VVA bindings in the testis from 16.5 dpc to 4.5 dpp. Arrowheads: gonocytes, small arrowheads: Sertoli cells, I: interstitium, T: testicular cord, S :seminiferous tubule. A: 16.5 dpc. VVA reacts only in a part of the interstitium. B: 0.5 dpp. VVA reaction is observed in the plasma membrane. C: 4.5 dpp. VVA reaction is observed in the plasma membrane and cytoplasm of gonocytes. D: Control section incubated with biotinyl VVA in the presence of 0.2 M N-acetylgalactosamine. Positive reaction is completely inhibited. × 480 each.

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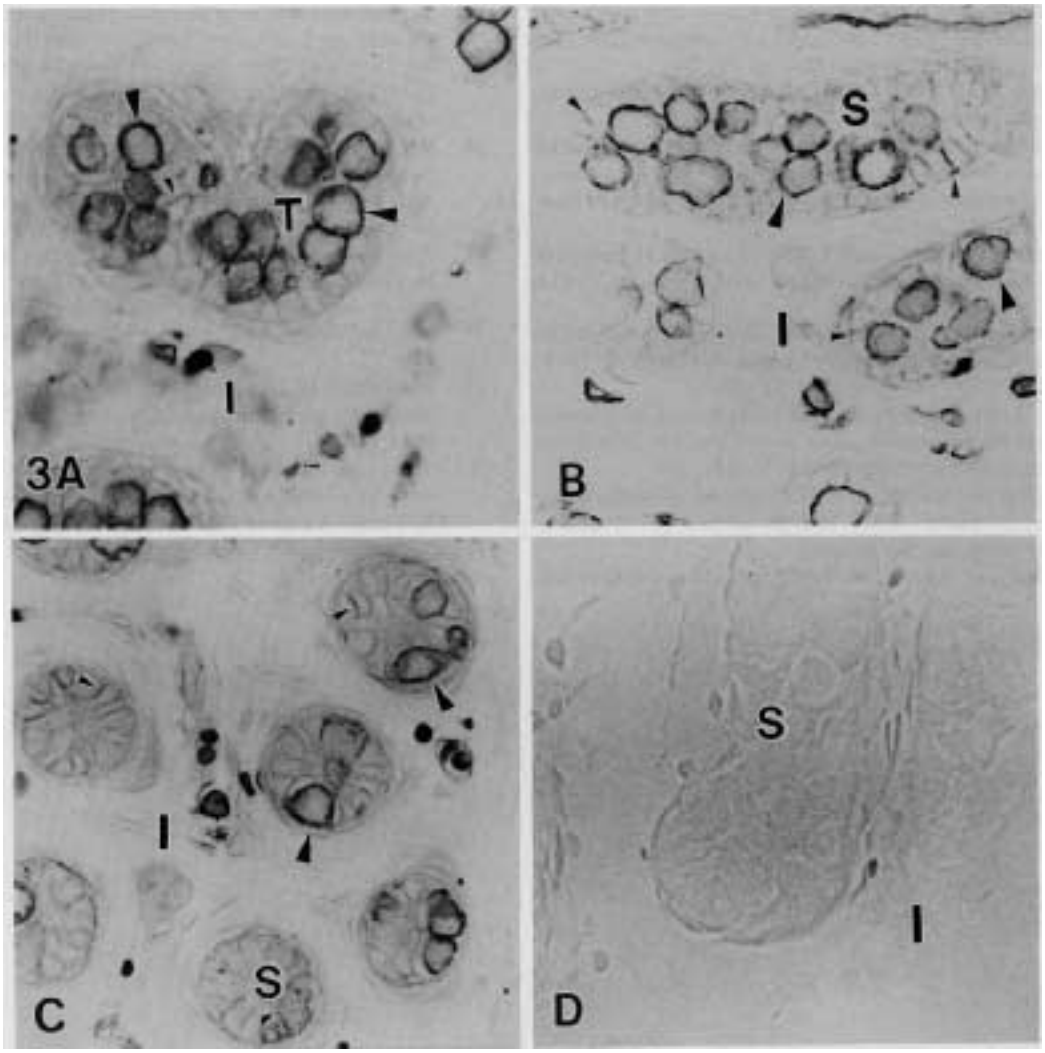

Fig. 3 A-D. Light micrographs showing LEA bindings in the testis from 16.5 dpc to 6.5 dpp Arrowheads: gonocytes, small arrowheads: Sertoli cells, I: interstitium, T: testicular cord, S: seminiferous tubule. A: 16.5 dpc. LEA reacts strongly in the plasma membrane and cytoplasm of gonocytes. B: 0.5 dpp. LEA is weakly positive only in the plasma membrane of Sertoli cells and a part of the basement membrane in addition to gonocytes. C: 6.5 dpp. LEA reacts strongly in the plasma membrane and cytoplasm in gonocytes. D: Control section incubated with biotinyl LEA in the presence of 0.2 M N-acetylglucosamine. Positive reaction is completely inhibited. × 480 each.

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