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Characterization of Lewis antigens in normal colon and gastrointestinal adenocarcinomas

(glycolipid antigens/monoclonal antibodies/cancer-associated antigen)

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ABSTRACT **The Lewis a antigen (Le^a)- and Lewis b antigen (Le^b)-active glycolipids and glycoproteins in normal colon mucosa, colon carcinoma tumors, and gastrointestinal tumor cell lines were studied by using monoclonal antibodies against Lewis determinants in solid-phase radioimmunoassay, chromatogram binding assay, and immunoprecipitation/PAGE. Le^a-5 was a major component of the purified non-acid glycolipid fraction from normal colon mucosas from four patients examined and Le^a-7 was a minor component in three of these four cases. Le^a in colon carcinoma of the same patients was expressed as Le^a-5 in all four cases and as both Le^a-5 and Le^a-7 in one of the four cases. All normal colon mucosa samples expressed Le^a but not Le^b. Le^b-6 was expressed in all tumors obtained from the same patients as normal colon mucosa (Le^b negative) samples and in the gastrointestinal tumor cell lines. These findings indicate that Le^b is a colon adenocarcinoma-associated antigen and that Le^a and Le^b are coexpressed in tumor tissue. Evidence is presented for the coexistence of Le^a and Le^b carbohydrate determinants in both glycolipid and glycoprotein forms in the same cells.**

Lewis blood-group antigen (Le) specificities reside in glycosphingolipids and glycoprotein molecules on the surface of erythrocytes (1) and epithelial and endothelial cells (2–4) and in bound or free glycoprotein oligosaccharides secreted into various body fluids, such as saliva, ovarian-cyst fluid (4), urine (5), and milk (6).

Lewis antigens in different gastrointestinal tumors have been studied by means of chemical and immunohistological techniques. Coexpression and an increase in the amount of Lewis blood-group antigen-active glycolipids have been found in human adenocarcinoma (7, 8) and, extremely rarely, in normal tissues (9). Breimer (10) detected the Lewis isoantigens Le^b-6 and Le^b-8 in the glycosphingolipid fraction derived from gastric adenocarcinoma of B blood-group patients. Siddiqui *et al.* (11) isolated Le^b-6 glycosphingolipid from cancer tissues but not from normal tissues. Hattori *et al.* (12) showed that Le^a-active glycolipid levels were higher but Le^b levels were lower in cancer tissues than in uninvolved tissue.

We report here the use of monoclonal antibodies in the biochemical characterization of the Le^a- and Le^b-active glycosphingolipid and glycoprotein antigens from the same cells. We found that Le^a and Le^b glycolipid antigens coexist in tumor cells, which is consistent with the results of chemical studies (8) and with observations based on immunoperoxidase techniques (13). The distribution of the Le^a and Le^b activities in the glycoprotein fraction corresponded well with that in glycolipids.

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MATERIALS AND METHODS

Monoclonal Antibodies. Le^a- and Le^b-specific antibodies CO-51.4 and CO-43.1, respectively, were produced by the hybridomas obtained after immunization of mice with colon carcinoma cell lines SW1116 and SW1222, respectively (37). P3×63Ag8 myeloma supernatant was used as a control. An anti-A blood group antibody, obtained from C. Civin (Johns Hopkins University), was used in one set of experiments (38).

Cells and Tissues. The following carcinoma cell lines were analyzed for expression of Le^a and Le^b antigens: one gastric (KATO-III), two pancreatic (CAPAN-2 and CH), seven colon (SW1116, SW948, SW1222, SW620, SW403, SW1345, and SW48), and one rectal (SW707) (39). Normal colon and colorectal adenocarcinoma specimens were obtained from the Fox Chase Cancer Center in Philadelphia.

Detection of Antigen Expression. Reactivity of the monoclonal antibodies that detect Lewis antigens with human gastrointestinal tumor cells was evaluated in radioimmunoassay (RIA) and in a mixed hemadsorption assay (MHA) as described (14).

Preparation and Analysis of Glycolipids. Normal colon mucosa, colon tumors, and cell lines were lyophilized, extracted with chloroform/methanol (2:1, vol/vol) according to the procedure of Folch *et al.* (15). The upper fraction was separated into ganglioside and non-acid glycolipids on a DEAE-Sephadex A-25 (H^+ form) column according to the method of Yu and Ledeen (16). The non-acid glycolipid fractions from the upper and lower phases were analyzed in solid-phase RIA and the chromatogram binding assay (17).

Analysis of Glycoprotein Antigen. Single-cell suspensions of normal colon mucosa were obtained by mincing specimens and incubating them with a solution of 0.14% collagenase (Sigma)/hyaluronidase (Sigma; 5 units/ml), and 0.025% DNase I (Sigma) in Eagle's minimal essential medium at 37°C for 30 min. Supernatants were collected after incubation and the procedure was repeated twice. Supernatants were centrifuged and the cell pellet was washed with Dulbecco's phosphate-buffered saline. Normal colon mucosa and colon carcinoma cell lines were labeled with ^{125}I by the lactoperoxidase method as described (18). Radiolabeled cells were solubilized with 50 mM Tris Cl buffer (pH 7.0) containing 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, and 0.2 mM phenylmethylsulfonyl fluoride for 30 min at room temperature and centrifuged at 100,000 × g for 1 hr. Immunoprecipitation and sequential immunoprecipitation procedures were done as described (19). $NaDodSO_4$/PAGE was in a 10% polyacrylamide gel under the conditions described by Laemmli *et al.* (20) and two-dimensional gel electrophoresis was as described by O'Farrell *et al.* (21). Dried gels were autoradiographed with Kodak film and DuPont intensifying screens.

Abbreviations: Le^a, Lewis a antigen; Le^b, Lewis b antigen; MHA, mixed hemadsorption assay.

Solid-Phase RIA. Solid-phase RIAs were performed with purified non-acid lipid fractions from normal colon mucosa, colon tumors, and carcinoma cell lines, according to the procedure of Brockhaus *et al.* (22).

Chromatogram Binding Assay. Non-acid lipid fractions were analyzed according to the procedure described by Hansson *et al.* (17).

RESULTS

Expression of Cell-Surface Antigens. Table 1 presents results from the MHA with the Le^a- and Le^b-specific monoclonal antibodies and gastrointestinal tumor cell lines. One gastric, one pancreatic, and four colon carcinoma cell lines were positive for both Le^a and Le^b. Three colon carcinoma cell lines and one rectal cell line showed no binding reactivity with either antibody. The same results were obtained in live-cell RIA (not shown).

Antigenic Activity of Glycolipid Fraction. To detect the Le^a and Le^b activities associated with glycolipids, the purified non-acid glycolipid fractions were analyzed with the Le^a- and Le^b-specific monoclonal antibodies in solid-phase RIA (Table 2). Le^a was expressed in all four normal colon mucosa samples, all four fresh colon tumors, and all but one of the seven cell lines. In contrast, Le^b was not detected in normal colon fractions but was expressed by all four fresh colon tumors and by all but one of the cell lines. The pattern of expression of Lewis antigens detected in MHA (Table 1) is consistent with results obtained by solid-phase RIA (Table 2), with the exception of SW707 rectal carcinoma cells, which were negative in MHA but positive for both Lewis antigens in RIA of the glycolipid fraction of the cell extract. The SW48 and SW1345 colon carcinoma cell lines, which did not express Lewis antigens in MHA, were not tested by the latter method.

Glycolipid Profile in Chromatography. To confirm the association of Lewis activities with glycolipids, non-acid glycolipid fractions were analyzed in a chromatogram binding assay. The glycolipid isoantigens that confer Lewis activities are represented in Fig. 1. The non-acid lipid fractions from four normal colon samples were reactive with the anti-Le^a (Fig. 1*A*) but not with the anti-Le^b antibody (Fig. 1*B*), in accord with the results in solid-phase RIA (Table 2). All fractions tested in the chromatogram binding assay showed a major band corresponding to Le^a-5 (Fig. 1*A*). The multiple, closely migrating bands at the Le^a-5 region probably reflect ceramide heterogeneity. One minor, slowly migrating band corresponds to Le^a-7. Slow-moving bands were not detected in glycolipids from three out of four colon tumors and most of the gastrointestinal-tract carcinoma cell lines. Le^a-active glycolipids from colon tumor sample CT-1 and from pancreatic carcinoma cell line CH showed the presence of an extended Le^a-7 structure. A band corresponding to a highly extended Le^a structure (more than 15 sugars) which did not migrate on the TLC plate was observed for the SW707 rectal carcinoma cell line. The non-acid lipid fractions from four

Table 1. Binding of anti-Le^a and anti-Le^b monoclonal antibodies to human gastrointestinal tumor cell lines in MHA

Cell line	Origin of adenocarcinoma	% positive cells*	
		CO-51.4 (Le^a)	CO-43.1 (Le^b)
KATO III	Stomach	80 ± 5	90 ± 5
CAPAN-2	Pancreas	20 ± 2	60 ± 3
SW403	Colon	80 ± 4	90 ± 5
SW948	Colon	60 ± 4	80 ± 5
SW1116	Colon	20 ± 3	60 ± 4
SW620	Colon	0 ± 2	0 ± 2
SW1345	Colon	0 ± 2	0 ± 2
SW1222	Colon	60 ± 3	80 ± 4
SW48	Colon	0 ± 2	0 ± 2
SW707	Rectum	0 ± 2	0 ± 2

*Mean ± SD, n = 4.

Table 2. Binding in solid-phase RIA of anti-Le^a and anti-Le^b monoclonal antibodies to glycolipid fractions from normal colon mucosa, colon tumors, and gastrointestinal tumor cell lines*

Tissue or cells	Origin*	Binding of monoclonal antibody, cpm	
		CO-51.4 (Le^a)	CO-43.1 (Le^b)
CN-1	Normal colon 1	3200	471
CN-2	Normal colon 2	4111	230
CN-3	Normal colon 3	3798	370
CN-4	Normal colon 4	3220	430
CT-1	Colon tumor of 1	4913	1650
CT-2	Colon tumor of 2	1300	920
CT-3	Colon tumor of 3	4150	2440
CT-4	Colon tumor of 4	3800	3400
KATO	Gastric carcinoma	5190	3520
CAPAN-2	Pancreatic carcinoma	1720	2840
CH	Pancreatic carcinoma	2080	1790
SW948	Colon carcinoma	4180	2880
SW1116	Colon carcinoma	2250	1680
SW620	Colon carcinoma	300	300
SW707	Rectal carcinoma	4580	3640

*Tumor and mucosa biopsies were obtained from each patient (numbers 1–4; see also Fig. 1).

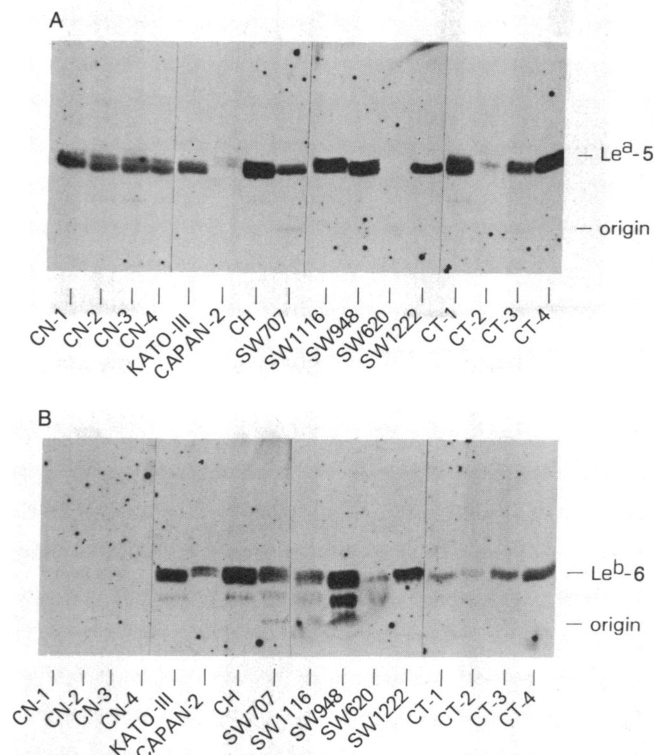

FIG. 1. Thin-layer chromatograms showing pattern of Le^a (*A*) and Le^b (*B*) in the glycolipid fractions obtained from normal colon mucosa, colon tumors, and human gastrointestinal tumor cell lines. Non-acid glycolipids corresponding to 1 mg of protein were chromatographed. Le^a and Le^b were detected by using monoclonal antibodies CO-51.4 and CO-43.1 as described (17). CN, normal colon mucosa (four individuals); CT, colon tumor (same four individuals).

colon tumors (corresponding to four normal samples) and from the cell lines CAPAN-2 and SW1222 resolved as a single band corresponding to Le^b-6 (Fig. 1*B*). The fractions derived from a fresh colon adenocarcinoma (CT-1) and from the KATO-III, CH, SW707, SW1116, and SW948 cell lines revealed faint, slow-moving bands of about eight sugars. Rectal carcinoma cell line SW707 and colon carcinoma cell line SW948 also showed the presence of Le^b-active structures of about 15 or more sugars, since they did not migrate on the plate.

Electrophoretic Profile of the Le^a and Le^b Glycoprotein Immunoprecipitates. Fig. 2 shows the electrophoretic pattern of Le^a- and Le^b-active glycoproteins immunoprecipitated from detergent extracts of ^{125}I-labeled cells of normal colon mucosa and gastrointestinal tumor cell lines. Two normal colon samples (from individuals A and B) showed the presence of Le^a-active glycoprotein of M_r 150,000 (lanes A2 and B2). Normal colon mucosa from individual A also expressed a glycoprotein of M_r 75,000 (lane A2). Again, Le^b-active glycoproteins were not detected in normal mucosa (lanes A3 and B3).

Most of the gastrointestinal tumor cell lines coexpressed Le^a- and Le^b-active glycoprotein(s) (Fig. 2). Both antigens were immunoprecipitated from CAPAN-2 pancreatic, SW707 rectal (not shown), SW1116 (Fig. 2) and SW1222 colon (not shown), and KATO-III gastric carcinoma cell lines. The SW948 colon carcinoma cell line was positive for both Le^a and Le^b in solid-phase RIA, but only Le^a glycoprotein was immunoprecipitated from cell line SW948 (Fig. 2). The SW620 colon carcinoma cell line was negative for both antigens, in accord with the results obtained for glycolipids (Fig. 1 *A* and *B*).

The glycoproteins isolated from most of the gastrointestinal tumor cell lines had similar apparent M_rs in $NaDodSO_4$/PAGE. The tumor cell lines revealed a M_r 150,000 component and a very high M_r component(s) that does not enter the separating gel. The glycoproteins from the SW1116 cell line were resolved as three bands (M_r 75,000, 80,000, and 85,000) when precipitated with either antibody (Fig. 2, SW1116 lanes 2 and 3).

Glycoproteins bearing Le^a and Le^b structures isolated from the same cells appear to have the same electrophoretic mobility.

Identification of Le^a and Le^b Glycoproteins from SW1116 Cells by Two-Dimensional Gel Electrophoresis. The surface membrane proteins from ^{125}I-labeled SW1116 cells were immunoprecipitated with anti-Le^a and -Le^b antibodies and subjected to two-dimensional gel electrophoresis. Virtually identical patterns were observed (Fig. 3). The protein spots corresponded to M_rs again in the range 75,000–85,000 and to pIs of 5.0–6.5.

The Le^a-active glycoproteins from normal colon (not shown) migrated in two-dimensional gel electrophoresis as a single, strongly acidic protein of M_r 150,000 (see also Fig. 2) and pI 3.2. The Le^b-active glycoprotein antigen was again absent.

Sequential Immunoprecipitation of Glycoprotein(s) with Le^a and Le^b Activity. Sequential immunoprecipitation analysis was performed to determine whether the Le^a and Le^b determinants are on the same molecule, which would explain the migration of the protein spots to identical M_r positions. SW1116 cells were found to have an A blood group phenotype in solid-phase RIA (data not shown). In the present experiments, an anti-A blood group monoclonal antibody was included together with anti-Le^a and anti-Le^b monoclonal antibodies. After immunoprecipitation of Le^a-active glycoprotein from the cell lysate, precipitation with the anti-Le^b antibody yielded no radioactive band (Fig. 4). When the antibodies were used in the reverse order, no radioactive band was obtained with anti-Le^a. These data indicate that the Le^a and Le^b carbohydrate determinants are associated with the same protein cores. The blood group A determinant is probably also associated with the same proteins as Le^a and Le^b, since the anti-A blood group antibody immunoprecipitated almost no material when preceded by anti-Le^a or anti-Le^b immunoprecipitation (Fig. 4). The second precipitation also gave no bands when immunoprecipitation with anti-A blood group antibody was followed by anti-Le^a or anti-Le^b precipitation.

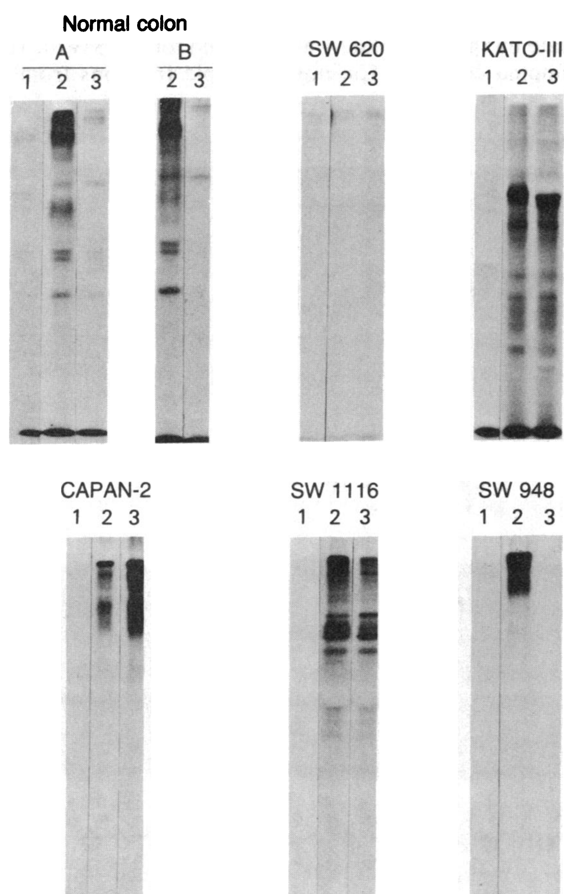

FIG. 2. Immunoprecipitation pattern of glycoproteins with Le^a and Le^b activities from normal colon mucosa of two individuals (A and B) and from gastrointestinal tumor cell lines. Immunoprecipitates were obtained with P×63Ag8 myeloma supernatant (lanes 1, control) and with monoclonal antibodies CO-51.4 (lanes 2, anti-Le^a) and CO-43.1 (lanes 3, anti-Le^b).

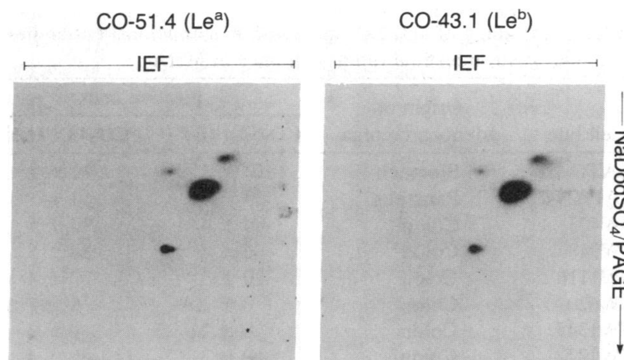

FIG. 3. Two-dimensional PAGE of Le^a-active and Le^b-active glycoprotein antigens immunoprecipitated from ^{125}I-labeled SW1116 cells. IEF, isoelectric focusing.

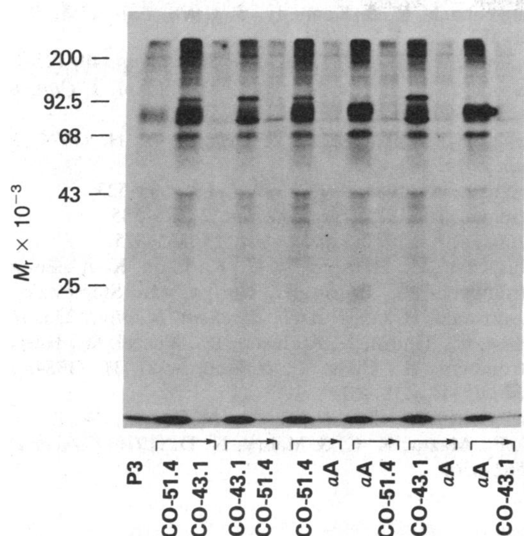

FIG. 4. Sequential immunoprecipitation of the antigens from ^{125}I-labeled SW1116 cells. Immunoprecipitation with supernatant from the P3×63Ag8 myeloma cells served as control (lane P3). Antibodies CO-51.4, CO-43.1, and *a*A are directed against Le^a, Le^b, and A blood group antigens, respectively. All lysates were absorbed with the first antibody and immunoprecipitated with the second antibody (sequence indicated by arrow) as described (18).

DISCUSSION

The Le^a and Le^b glycolipid antigens were coexpressed in human adenocarcinoma tissues and tumor cell lines in 10 out of 14 cases as detected by solid-phase RIA. The original Lewis phenotype and secretor status of the patients were not available. Le^b was expressed in each of the four colon tumor samples but not in the normal colon mucosa obtained from the same respective patients.

Ernst *et al.* (13) detected Le^b in 73% of distal colon carcinomas by using an immunoperoxidase staining technique. Normal tissue from the distal colon expressed only Le^a. Based on those results, we assume that the normal colon and colon tumor samples used in the present study derived from distal colon. All gastrointestinal tumor cell lines that were positive for Lewis antigens coexpressed both Le^a and Le^b, in accord with previous observations (7, 8, 13).

In the chromatogram binding assay of the Le^a-active glycolipids, the slow-moving bands representing the extended structures of the antigen were absent in three out of four colon tumor samples, whereas their normal counterparts contained these structures. Five out of seven of the tumor cell lines also lacked these extended structures. These findings are consistent with the reported diminished capacity of some transformed cells (23–25) and tumors (26, 27) to extend the carbohydrate chains of their glycolipids. The suppression of specific glycosyltransferases might account for the incomplete synthesis of higher glycolipids (28–30).

The Le^b blood group activity in tissues is under the control of the *Se* (secretor) gene and is a gene-interaction product of *Se* and the Lewis gene *Le* (31). The expression of Le^a but not Le^b in normal colon tissue suggests that the *H* gene, which encodes an Le^b precursor and is controlled by the *Se* gene, is inactive, while the *Le* gene remains able to encode Le^a. It is possible that in individuals who are genotypically either *Lele* or *LeLe*, the transformation process activates a regulatory function of the *Se* gene over the *H* gene or directly induces the *H*-encoded ($\alpha 1 \rightarrow 4$)fucosyltransferase. The ($\alpha 1 \rightarrow 4$)fucosyltransferase, which acts on a lacto-*N*-tetraosylceramide resulting in *H* type I substance, is influenced by *Se* or is induced directly in the process of malignancy and results in biosynthesis of the Le^b associated with colon adenocarcinoma.

The coexistence of glycolipid forms of both Le^a and Le^b and not of Le-active glycoprotein in the gastrointestinal tumor cell lines tested indicates that the attachment of the Le-active oligosaccharides to a ceramide and the protein core proceeds by two independent enzymatic pathways. The core polypeptide for Le^a, Le^b, and the blood group A oligosaccharide haptens is the same, as demonstrated by sequential immunoprecipitation.

The availability of monoclonal antibodies specific for Lewis and other blood-group antigens has enabled elucidation of their long-debated chemical nature. We have shown that the oligosaccharide haptens of Le^a and Le^b phenotypes are expressed on both glycolipid and glycoprotein moieties in gastrointestinal tumors. The expression, distribution, and involvement of blood-group antigens in the process of malignancy can now be studied further using monoclonal reagents. Some relationship between Le^b and blood-group antigens of the ABO system can be drawn in this respect. ABO antigens are not detectable in normal mucosa of distal colon but were found in adenocarcinomas of this region by immunocytochemical methods (32, 33, 38). Also, ABO and Lewis antigens appear at 8 weeks of gestation and are suppressed after birth (34–36). Their reexpression in tumors of distal colon mimics the situation in embryonal life; thus Le^b and ABO substances in tumors of this part of the colon may be considered to be of oncodevelopmental type.

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