been classified mainly into two types, namely the surface mucous cells and the gland mucous cells. Ota *et al.* indicated by a histochemical staining method that the mucins accumulated in and/or secreted from these two types of cells differ from one another in the carbohydrate moiety (1). To discriminate the type of mucin, we attempted to raise a monoclonal antibody (MAb) which recognizes the particular oligosaccharide structure. For the purpose of immunization of mouse and characterization of the MAb, a mucin was separated from the surface mucous cells of rat stomach using Bio-Gel A-1.5 m column chromatography and two steps of CsCl equilibrium centrifugation. A MAb, designated RGM11, was generated by the fusion of the splenic lymphocytes of immunized mouse with Sp2/0-Ag14 mouse myeloma cells. RGM11 reacted with the purified mucin which had been attached to the ELISA well. This reaction was inhibited by the oxidation of the ELISA well with periodate, indicating the carbohydrate moiety of the mucin molecule to be the epitope of RGMI1. Treatment of the ELISA well with galactose oxidase also reduced the reaction with this MAb, thus suggesting the peripheral galactose and/or *N*-acetylgalactosamine residues of the carbohydrate moiety of mucin are involved in the epitope structure. Immunohistochemical observation indicated that this MAb was able to stain the formalin fixed-paraffin embedded sections of rat and was positive to the surface mucous cells of corpus and antral region of the stomach and the villus epithelium of the duodenal mucosa, but other organs and tissues of rat so far examined were all negative to this MAb. These results indicate that the newly established MAb, RGM11, might be useful to estimate the physiological and pathological changes in gastric surface mucous cells and the mucin derived from these cells.

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S20.14

A Monoclonal Antibody, RGM41, Specific for the Mucin Derived from the Gland Mucous Cells of Mammalian Stomach

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The mammalian gastric gland mucous cells (GMC), including mucous neck and pyloric gland cells, are synthesizing and secreting mucin species, which can be detected by a unique histochemical method, the paradoxical concanavalin A staining (PCS) (1). Mucus of GMC is suggested to have a distinct role in the gastric mucosal defence mechanism (2). While a biochemical study has been done to justify the sequence of PCS reaction on the purified gastric mucin (3), no specific carbohydrate structure responsible for the PCS has yet been clarified. In this study, we attempted to raise a monoclonal antibody (MAb) which recognizes the particular oligosaccharide structure present in the mucin derived from the gastric GMC. For this purpose, gastric mucin obtained from the whole layer of rat gastric mucosa was purified by a Bio-Gel A-1.5 m column chromatography and two steps of CsCl equilibrium centrifugation, and utilized for the immunization of mouse and the characterization of MAb. Several MAbs were generated by the fusion of the splenic lymphocytes of immunized mouse with Sp2/0-Ag14 mouse myeloma cells. These MAbs reacted with the purified mucin which had been attached to the ELISA well. Immunohistochemical observation indicated that one of these MAbs, designated RGM41, was specific to the GMC of corpus and antral region of rat, human and porcine gastric mucosa. The reaction of RGM41 with the purified mucin was not inhibited by the pretreatment of the ELISA well either with the periodate oxidation or with trypsin digestion. But this MAb reacted strongly with the oligosaccharide mixture obtained from the purified mucin by alkaline borohydride reduction followed by Toyopearl HW-50S chromatography. These results indicate that the newly established MAb, RGM41, might recognize a particular carbohydrate structure which is specifically involved in the mucin derived from the gland mucous cells of mammalian gastric mucosa.

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S20.15

Novel Monoclonal Antibody Recognizing the MUC1 Apoprotein Among Highly Glycosylated Sialyl-Le^a Expressing Glycoproteins from Colon Carcinoma Cells

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Previous studies on glycoproteins from the colon carcinoma cell line COLO 205 carrying the carcinoma-associated sialyl-Le^a carbohydrate epitope have revealed that sialyl-Le^a is present both on the MUC1 epithelial mucin and on another, smaller apoprotein. Comparing the situation in COLO 205 cells with that of other cell lines was facilitated by the use of a novel MUC1-reactive monoclonal antibody, Ma552, raised against a mammary carcinoma cell line.

Ma552 recognizes the hexapeptide TRPAPG located within the tandem repeat sequence of the MUC1 protein. It was capable of binding to H-CanAg, the MUC1 mucin from the COLO 205 cell line, in spite of the fact that this mucin is heavily glycosylated (85% carbohydrate by weight). In contrast, the monoclonal antibody HMFG-2, which recognizes the MUC1 protein in mucins from breast epithelium (which are less glycosylated), failed to bind intact H-CanAg without prior deglycosylation.

In order to study the sialyl-Le^a carrying glycoproteins of the four colon carcinoma cell lines COLO 205, SW1116, LoVo, and LS174T, gel filtration fractions of cell extracts and spent tissue culture media were analyzed in two immunofluorometric assays. In a homologous assay in which the sialyl-Le^a reactive monoclonal antibody C50 was used both as catching and tracing antibody, all sialyl-Le^a carrying glycoproteins were detected. Among these, mucins based on the MUC1 apoprotein were singled out in a heterologous assay using Ma552 as catching antibody and C50 as tracer. The results revealed size