EFFECTS OF GASTRIC ANTIBODIES ON GASTRIC SECRETION

I. PRODUCTION OF RABBIT ANTIBODIES AGAINST RAT GASTRIC MUCOSA AND GASTRIC JUICE

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It has been shown that rabbits injected with a homogenate of rat gut mucosa produce auto-antibodies reacting with their own gut mucosa (1). Hausamen et al. (2) have demonstrated that the injection of an aqueous extract of guinea pig stomach into rabbits leads to the production of hetero- and auto-antibodies against parietal cell antigen, gastric intrinsic factor and mucous substances confined to the stomach. Hennes et al. (3) reported that antibodies, reacting with gastric juice and an extract of dog gastric mucosa, were found in dogs immunized with gastric juice from various sources. This paper describes the production of gastric antibodies to rat gastric antigens by the injection of rat gastric mucosa and gastric juice into rabbits, and characterization of these antibodies.

MATERIALS AND METHODS

Animals

Male white rabbits, over 2.5 kg were randomly assigned to the different experimental groups.

Preparation of antigens

Gastric mucosa: Gastric mucosal antigen was obtained from rats which had been given tetracycline in their drinking water for 5 days, and then only glucose solution for 24 hr, before being sacrificed. Stomachs were removed immediately after death, opened, washed in ice cold phosphate buffered saline and mucus was removed by blotting on filter paper. The mucosa of the oxyntic area of the stomach was scraped off and collected. The mucosal scrapings were mixed with 2 volumes of phosphate buffered saline and homogenized in a glass tube with a Teflon pestle for 5 min at high speed. After overnight storage at -20°C , the homogenate was thawed and centrifuged at 6,000 g for 30 min. The supernatant was used as source of antigen. Protein concentration, determined by biuret analysis, was 17.5 mg/ml.

Gastric juice: Fresh tetragastrin-stimulated gastric juice was collected into the ice cold reservoir from rats with chronic fistulae. The gastric juice was centrifuged at 6,000 g for 30 min, and the clear supernatant dialyzed in a cellulose tube against a large volume of phosphate buffered saline, and then lyophilized. Thirty mg of the lyophilized material

(containing 4 mg of protein) was dissolved in 1 ml of phosphate buffered saline.

Uterus: The uterus was removed from the rat, immediately after sacrifice and washed in phosphate buffered saline. The organ was minced with scissors, ground in a double amount of phosphate buffered saline in a Waring blender for 5 min at moderate speed, and then homogenized. Further procedures were carried out as described in the preparation of the gastric mucosal antigen.

Immunization

The above antigen preparations were homogenized with an equal volume of complete Freund's adjuvant (Difco Laboratories) to give a stable emulsion. One milliliter of this emulsion was injected intramuscularly into each of the four footpads, the extremities and subcutaneously at several sites on the backs of rabbits, at bi-weekly intervals. From 3 to 7 months after the first immunization, the animals were bled from the ear one week after each booster injection. The sera from all animals within a group were pooled and used for the antibody titration, immunodiffusion analysis, as well as for the preparation of γ -globulin.

Antisera absorption

The antisera were incubated with equal volumes of normal rat serum and lyophilized homogenate of rat esophagus for 30 min at 37°C, and then allowed to stand overnight at 4°C. After centrifugation for 30 min at 6,000g to remove resultant precipitates, the antisera were tested by antibody titration and immunodiffusion analysis.

γ -Globulin preparation

Pooled antisera were fractionated at a final concentration of 33% saturated ammonium sulfate, the precipitate being taken up in phosphate buffered saline, dialyzed against a large volume of the same solution and lyophilized. The γ -globulin fractions were stored at 4°C and used for the direct immunofluorescence test and the pepsin activity inhibition test.

Antibody titration

Twofold progressive dilutions of the antisera were made in 0.2 ml of phosphate buffered saline. Equal volumes of the antigen, at an optimum concentration, were added to each tube. The tubes were agitated, then left for 2 hr at 37°C and overnight at 4°C. Contents of the tubes were inspected for precipitates after 2 hr at 37°C and after the reaction mixtures were left overnight in a refrigerator. The last tube showing a definite sediment was taken as an end point, and the precipitin titer was expressed in terms of a final anti-serum dilution in this tube.

Immunodiffusion (Ouchterlony test)

Immunodiffusion analysis was performed in a 0.5% agar medium prepared in a barbital buffer, pH 8.6, ionic strength 0.05.

Direct immunofluorescence test

The direct immunofluorescence test was performed by the method modified by Kawamura (4) on 7 μ cryostat sections obtained from the fundic portion of the stomach and the colon of the rat. The sections were incubated overnight at 4°C with fluorescein isothiocyanate labeled γ -globulins, washed thoroughly in phosphate-buffered saline for

15 min, covered with buffered glycerol and examined by ultra-violet microscopy (TRYDA FM-200).

Pepsin activity inhibition

The pepsin activity was determined by a modification of Bock's method (5) using bovine serum albumin (The Armour Laboratories) as a substrate. For determination of the inhibition of pepsin activity by the antibodies, diluted homogenate of rat gastric mucosa and gastric juice were incubated with increasing amounts of γ -globulin preparations, obtained from antisera against rat gastric mucosa and gastric juice, respectively, at 37°C for 30 min. Subsequently, they were centrifuged at 3,000 r.p.m. for 15 min to remove the resultant precipitates, after which they were mixed with the substrate, incubated at 37°C for 30 min and the pepsin activity measured.

RESULTS

Twelve rabbits, divided into three groups of 4 animals, were immunized with rat gastric mucosa (GM), rat gastric juice (GJ), or rat uterus (Ut). The pooled antisera obtained from the three groups of rabbits were tested for their antibody titer against the corresponding group antigen, and in immunodiffusion analysis against the different group of antigens. The results are summarized in Table I.

The precipitin titers of GM sera, GJ sera and Ut sera were 1 : 32, 1 : 16 and 1 : 16,

TABLE I. Antibody titers and immunodiffusion analysis of rabbit antisera immunized with rat gastric mucosa (GM), gastric juice (GJ) and uterus (Ut).

No. of rabbits	Immunized with	Precipitin titer	Immunodiffusion analysis antigens		
			GM	GJ	Ut
4	GM	1 : 32	—	—	—
4	GJ	1 : 16	—	—	—
4	Ut	1 : 16	—	—	—

FIG. 1. Immunodiffusion analysis of rat gastric mucosa homogenate (1) with rabbit antisera against rat gastric mucosa (2) and rabbit antisera against rat uterus (3) in agar medium.

FIG. 2. Immunodiffusion analysis of rat gastric juice (1) with rabbit antisera against rat gastric juice (2) and rabbit antisera against rat uterus (3) in agar medium.

respectively.

In immunodiffusion analysis, GM sera and GJ sera formed precipitin lines against GM and GJ (Figs. 1 and 2). When GM sera and GJ sera were tested against GM (Fig. 3) and GJ (Fig. 4), precipitin lines of identity between the two antisera were observed. Ut sera

FIG. 3. Immunodiffusion analysis of rat gastric mucosa homogenate (1) with rabbit antisera against rat gastric juice (2) and rabbit antisera against rat gastric mucosa (3) in agar medium. Note the lines of identity between the two antisera.

FIG. 4. Immunodiffusion analysis of rat gastric juice (1) with rabbit antisera against rat gastric juice (2) and rabbit antisera against rat gastric mucosa (3) in agar medium. Note the lines of identity between the two antisera.

FIG. 5. Section of normal rat stomach stained immunofluorescent with rabbit antibodies against rat gastric mucosa absorbed with normal rat serum and esophagus homogenate. A, note the staining of nearly all the cells of the gastric mucosa ($\times 100$). B, note the bright fluorescent staining of the parietal cell cytoplasm ($\times 400$).

FIG. 6. Section of normal rat stomach stained immunofluorescent with rabbit antibodies against rat gastric juice absorbed with normal rat serum and esophagus homogenate. A, ($\times 100$). B, note specific fluorescence of the mucus and the mucus-containing cells ($\times 400$).

did not react with either GM or GJ (Figs. 1 and 2).

In the direct immunofluorescence test, GM sera gave a definite cytoplasmic staining in nearly all the cells of the gastric mucosa (Fig. 5A). The intensity of fluorescence of the parietal cell was strong (Fig. 5B), but was weak in the peptic cell. GJ sera gave bright, specific fluorescence of the mucus on the surface of the gastric mucosa, within the gastric pits, in cells of the surface epithelium and in the peptic cell (Fig. 6A and 6B). The parietal cell was stained very weakly. On the other hand, Ut sera did not react with the rat stomach (Fig. 7). Colon from the rat showed no fluorescence with these antisera.

In the study of pepsin activity inhibition, GM antibodies were capable of inhibiting the pepsin activity of homogenate of rat gastric mucosa (Fig. 8), and GJ anti-

FIG. 7. Section of normal rat stomach stained immunofluorescent with rabbit antibodies against rat uterus absorbed with normal rat serum and esophagus homogenate ($\times 100$).

FIG. 8. Inhibitory effect of increasing amounts of rabbit γ -globulin preparation against rat gastric mucosa on pepsin activity of rat gastric mucosa homogenate.

FIG. 9. Inhibitory effect of increasing amounts of rabbit γ -globulin preparation against rat gastric juice on pepsin activity of rat gastric juice.

bodies inhibited the pepsin activity of gastric juice (Fig. 9). These inhibitions were found to be dose-dependent. This demonstrates that GM antibodies and GJ antibodies contain antibodies against pepsin and pepsinogen.

DISCUSSION

It is well known that rabbits immunized with heterologous antigens of the gastrointestinal tract will produce hetero- and auto-antibodies against the components of the tissues used as antigens (1, 2, 6). The present study also demonstrates that hetero-antibodies against an aqueous extract of rat gastric mucosa and rat gastric juice were produced in the rabbit.

Hausamen et al. (2) demonstrated that rabbit antisera against guinea pig gastric mucosa contained organ-specific antibodies which showed immunofluorescent staining of the parietal cell cytoplasm, surface epithelial cells, mucous neck cell regions and mucous substances confined to the stomach. The pattern of staining resembled that given by GM antibodies in this study. Weak immunofluorescent staining with GJ antibodies also was noted in the parietal cell. These results indicate that GM antibodies and GJ antibodies may contain antibodies against the parietal cell components.

Pepsin and pepsinogen are known to have antigenicity (7, 8). In the immunofluorescence test, GM sera gave a weak fluorescent staining of the peptic cell, while GJ sera gave a definite fluorescent staining. This is in good agreement with the results of the pepsin activity inhibition by these antibodies, as GJ antibodies inhibited the pepsin activity more strongly than did GM antibodies. These findings demonstrate that both GM sera and GJ sera contain antibodies against pepsin and pepsinogen. From these data, it is suggested that at least one of the lines of identity observed between GM sera and GJ sera in the immunodiffusion analysis may be antibodies against pepsin and pepsinogen.

It has been shown that antibodies against gastrointestinal extracts are mainly directed to the substances present in both the secretions and cytoplasm of mucus-producing cells (2). Antibodies against gastric mucus, which are present in mucous neck cells as well as in cells of the gastric surface epithelium and their secretions, were also found in GM sera

and GJ sera by the immunofluorescence test. These antibodies may be responsible for the lines of identity observed in the immunodiffusion analysis.

In the present study, antibody against gastric intrinsic factor was not examined, but Hausamen et al. (2) showed that rabbits injected with aqueous extracts of adult guinea pig stomach produced antibodies against gastric intrinsic factor.

Absorption of antisera with normal rat serum and homogenate of esophagus seems to be sufficient to remove nonspecific antibodies, as Ut sera did not react with either rat gastric mucosa, gastric juice or colon. In addition, GM sera and GJ sera did not react with colon.

SUMMARY

Organ specific hetero-antibodies were produced by immunizing rabbits with rat gastric mucosa and gastric juice. Common antibodies were found in antisera against rat gastric mucosa and gastric juice. Antibodies against gastric mucosa were mainly directed to the parietal cell, constituents of the mucus-producing cells and slightly to the peptic cell, while antibodies against gastric juice were mainly to the mucus-producing cells, their secretions, the peptic cell, and slightly to the parietal cell. These antibodies inhibited in vitro the pepsin activity of a homogenate of gastric mucosa and gastric juice.

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