INDUCTION OF AUTOALLERGIC GASTRITIS IN DOGS

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INTRODUCTION

Reports of the experimental production of autoallergic gastritis have been contradictory. Intravenous injection of human gastric juice into dogs was shown to have an immediate inhibitory effect on gastric secretion by Brunschwig et al. (1939). Subsequently, Smith, Joel and Wolf (1958), and Smith et al. (1958), described histologic gastritis as well as decreased secretion in dogs following intravenous injection of human gastric juice. Later, the same group (Hennes et al., 1962), reported the production of atrophic gastritis following the injection of gastric juice with Freund's adjuvant into dogs, and suggested an immunologic mechanism as the basis for these findings. Fixa et al. (1964), confirmed these findings. However, Sircus et al. (1963), using the same methods, were unable to demonstrate either histologic gastritis or a decrease in gastric physiologic function. Further attempts to produce autoallergic gastritis in rats (Fisher and Taylor, 1972) and guinea-pigs (Roitt, Doniach and Shapland, 1965) have also met with little success.

In man chronic gastritis has been shown to be associated with the presence of parietal cell specific auto-antibodies. There has been speculation that these antibodies perpetuate or perhaps cause gastritis in man (Bauer, Roitt and Doniach, 1965; Fisher and Taylor, 1965; Irvine et al., 1965). Recently, the successful demonstration of histologic gastritis and sera which contained parietal cell specific auto-antibodies was reported by Krohn (1968) in dogs, and Andrada, Rose and Andrada (1969) in monkeys. The former injected either canine gastric juice or gastric mucosa mixed with Freund's adjuvant, and the latter utilised homologous gastric mucosa and adjuvant.

These reported successes rekindled our enthusiasm to create a reliable experimental model for investigation of the phenomenon of autoallergic gastritis. In addition to the histologic and immunofluorescent studies of Krohn, we have added the evaluation of physiologic function as manifested by Heidenhain pouch secretion, and serologic evaluation by immunodiffusion and complement fixation.

METHODS

A. Preparation of dogs

Following a 2-wk period of observation and acclimation, Heidenhain pouches with indwelling metal cannulas were constructed under barbiturate anesthesia in nine mongrel dogs ranging in weight from 11.4 kg to 16.8 kg. The dogs were fed on a standard kennel diet.
dict. After a 4-wk interval, base-line studies of maximal pouch secretion were made in all dogs in the following manner. After 16 hr of fasting, histamine, 0.35 mg/kg (Baron et al., 1965) was injected subcutaneously and secretions were collected over a 90-min. period. Each dog was tested every other day for three or four tests. After recording volume and measuring the pH on a Radiometer pH meter, the m.equiv. of H+ secreted were calculated by titration with 0.1N-NaOH to pH 7. From the outset of immunisation, secretory studies were performed as described above at 4-wk intervals. Mean values and their standard errors were calculated for each set of tests.

B. Preparation of the antigens

1. Gastric mucosa antigen. Four dogs were acutely exsanguinated. The stomachs were immediately excised, and the antrums were discarded. The fundi were washed with iced physiologically buffered aline (PBS) at pH 7.2 and full thickness sections were taken for histology. The mucosa was then separated and mixed with a 0.1 per cent. fician solution (VSB) and digested for 50 min. at 4°C, followed by centrifugation of the tissue suspension at 2000 r.p.m. for 35 min. The washing process was repeated. The sediment was then homogenised in a Potter-Elvehjem homogeniser with added PBS at pH 7.2. The homogenate was centrifuged at 5000 r.p.m. for 30 min. and the supernatant was dialysed against distilled water at 4°C over 4 hr, changing the dialysate every 30 min. The supernatant was then lyophilised and stored at -20°C. The antigen preparation contained 0.937 mg protein/mg powder, as determined by the Lowry method.

2. Gastric juice antigen. Following a subcutaneous injection of histamine, pouch secretions were collected from six dogs at 15-min. intervals over a 90-min. period, and were immediately depepsinised by raising the pH to 10 for 30 min. with 1N-NaOH. Secretions directly from the stomach were found unsuitable due to high content of foreign material. Following depepsinisation, the fluid was filtered through #40 Whatman filter paper and adjusted to pH 7.2 with 1N-HCl, and then centrifuged at 3500 r.p.m. for 30 min. The supernatant was dialysed against PBS at pH 7.2 for 4 hr at 4°C, changing the dialysate bath every 30 min. Concentration was performed by ultrafiltration at 4°C, such that 1 ml of concentrate was the equivalent of 50 ml of gastric pouch juice, and then stored at -20°C. A Lowry protein determination on the aliquot showed 15.375 mg protein/ml.

C. Immunisation of the Dogs

The dogs were randomly divided into two experimental groups, those receiving gastric mucosa or gastric juice, and the control group. The antigens were mixed with an incomplete Freund’s adjuvant (Difco) to which was added M. tuberculosis at a concentration of 8 mg/ml. Four dogs were immunised with gastric mucosa, and received 100 mg of lyophilised powder dissolved in 1.5 ml normal saline, and emulsified with 0.75 ml of adjuvant. Two dogs were immunised with 1 ml of gastric juice concentrate and 0.5 ml of adjuvant. Immunisations were performed at 2-wk intervals via multiple, small intradermal, subcutaneous, and occasionally, intramuscular injections.

D. Immunologic studies

Serum samples were taken from each dog prior to pouch construction and at 2-wk intervals after the onset of immunisation. Three tests for gastric antibodies were used:

1. Immunofluorescence. Dog sera were tested by the indirect Coon’s immunofluorescence technique (Coons and Kaplan, 1950), using 6μm frozen sections of dog, hog and human stomach, and fluorescein-conjugated rabbit, anticanine gammaglobulin from two separate sources (Hyland and Pentex laboratories). Rhodamine-labelled bovine serum albumin was also employed, using the sera that gave precipitating lines in gel diffusion. Each serum was directly conjugated with fluorescein and then applied to 6μm sections of the above tissues.

2. Immunodiffusion. Each serum sample was tested by the Ouchterlony method against both gastric mucosa and gastric juice antigens.
3. Complement fixation. Both juice and mucosal preparations described above were used as the antigen in tests performed by the semimicro method of Donnelly (1951), using twice the minimal hemolytic dose (MHD) of complement.

E. Histology

Control gastric biopsies were taken from each dog at the time of pouch construction. Following the onset of immunisation, biopsies of the stomach were performed at the 10th, 20th and 30th wk. Biopsies of the pouch were also performed in most instances. The biopsies were fixed in 10 per cent. formalin and stained with both haematoxylin and eosin and phosphotungstic acid haematoxylin. These biopsies were then evaluated, blindly, for the degree of inflammation, oedema, fibrosis and glandular atrophy.

RESULTS

Of the nine dogs in the study, four survived for 30 wk after the onset of immunisation. Two of these were control dogs and two were dogs immunised with gastric mucosal antigen. All of these dogs either maintained their pre-study weights or gained weight throughout the study. The two dogs receiving
gastric juice antigen died during the 11th and 19th wk post-immunisation following an unrelenting 2-wk downhill course of intermittently bloody diarrhoea, anorexia and weight loss. Stool and haematologic examinations for suspected parasites were negative; however, the dogs were empirically treated with pyrimethamine without improvement. At the time of death petechial haemorrhages through the mucosa of the distal small bowel and colon were observed. The stomach appeared grossly normal. Autolysis precluded the assessment of histology. Of the other three dogs who failed to complete the course of the study, one was a control dog and two were receiving the gastric mucosal antigen. These died at 5, 10 and 11 wk, respectively, following a 3-wk period of anorexia and weight loss. They did not have diarrhoea. At the time of death, the stomach and intestines appeared grossly normal.

Secretory studies of the Heidenhain pouches in the three control animals following stimulation with a maximal dose of histamine, showed no alteration of acid output throughout the course of the study (fig. 1). Those dogs receiving gastric juice antigen also failed to show a reduction in acid output (fig. 2). Of the four dogs receiving gastric mucosal antigen, all showed a decrease in acid
output which was, however, only significant in those two dogs that survived for the duration of the study (fig. 3).

The serum from one of the test dogs receiving the mucosal antigen (#6) demonstrated a precipitin line against the antigen by immunodiffusion on the 8th wk post-immunisation. Subsequent sera continued to demonstrate this reaction throughout the remainder of the study. There was no cross-reaction
against the gastric juice antigen. The same sera, however, failed to show positive immunofluorescence by either direct or indirect methods on canine gastric mucosa, or heterogenous tissue. Complement fixation using gastric mucosal extract gave negative results. The sera from all other dogs showed no positive reaction utilising immunodiffusion, complement fixation and immunofluorescence.

The results of the histologic evaluation are summarised in the table (fig. 4). On the basis of histological parameters, we were unable to demonstrate significant pathologic differences between either of the test groups and the control group. Inflammatory and degenerative changes were found to a comparable degree in biopsies of both the test groups and the control group on the initial biopsy prior to immunisation. In addition, throughout the duration of the immunisations, there was no significant increase in atrophy, fibrosis or inflammation. Finally, the histologic patterns in the stomach biopsies were not significantly different from those of the pouch biopsies.

DISCUSSION

Parenteral immunisation of dogs with gastric mucosa and gastric juice, both emulsified in Freund's adjuvant, failed to yield a consistent response as measured by the presence of circulating antibody. The serum of only one of four dogs immunised with gastric mucosa demonstrated precipitating antibody against a mucosa antigen at 8 wk and throughout the remainder of the study, and this could only be demonstrated by gel diffusion. Interestingly, this same dog, along with the three other dogs receiving the gastric mucosal antigen, demonstrated a depression of maximal Heidenhain pouch acid secretion. However, the decrease was only significant in those two dogs who survived the duration of the study. The weights of these two animals remained stable throughout the study. Neither those dogs receiving the gastric juice antigen, nor the control dogs demonstrated evidence of a circulating antibody or diminished secretion.

It is difficult to account for the failure to detect a circulating gastric antibody in more of the test dogs. It can be hypothesised that circulating antibodies may have been masked by antigen excess. We did not look for the local production of antibodies in the gastric mucosa. It is known, however, that in atrophic gastritis in man, local antibodies against components of the parietal cell can be identified in plasma cells in the gastric mucosa, as well as in the serum.

We could not demonstrate significant inflammatory or atrophic changes to correlate with the prominent drop in histamine-stimulated acid secretion in the dogs receiving the mucosal antigen. In fact, the mucosal histology did not differ between test and control dogs. The biopsies from all dogs showed either: (1) mild chronic inflammation, oedema and lymphoid follicles; or (2) a moderate chronic inflammatory infiltrate with occasional polymorphonuclear leucocytes and increased amounts of connective tissue. The former histologic picture has been referred to by previous authors as a positive result, induced by their experimental method (Smith et al., 1958; Hennes et al., 1962; Krohn et al., 1968). However, we found this histologic picture in the pre-immunisation
biopsies of our control and test dogs with normal pouch secretion and normal serology, and similar findings have been described in a standard histology test of domestic animals (Trautman et al., 1957). These histologic findings can be focal as well as diffuse and this raises the question of error on random biopsy. Mucosal thickness was an unreliable indicator of atrophy due to shrinking of the mucosa and contraction of the muscularis mucosae prior to fixation.

The simple histology of this study may not have been enough to detect mucosal changes occurring as a result of autoimmunisation. The duration of the experiment may have been too short, as the time needed to develop gastritic changes is not known. Furthermore, in man, we do not know the interval between chronic gastritis and atrophy or even whether the former progresses to the latter.

The significant conclusion from this part of the study is that care must be taken to establish a representative histologic base-line prior to any attempts at production of autoallergic gastritis.

**SUMMARY**

In the past, reports of the experimental production of autoallergic gastritis have been contradictory. An attempt has been made to develop a functional experimental model of autoallergic gastritis. Test dogs were injected with either a homologous preparation of gastric juice or gastric mucosa, emulsified with Freund’s adjuvant. Two of the four dogs receiving the gastric mucosal antigen demonstrated a significant decrease in acid output from Heidenhain pouches following maximal stimulation with histamine. Sera from one of the dogs formed precipitin lines against the antigen on immunodiffusion. Neither of these features was demonstrated by the control dogs or those immunised with gastric juice preparation. Neither were antibodies found in sera tested by immunofluorescence and complement fixation. Histologically, there was no significant difference between control and test dogs, and the histologic findings did not correlate with the functional studies. These findings are discussed and the need for control studies to evaluate histological changes is stressed. It is concluded that parenteral immunisation with a gastric mucosal homogenate produces decreased acid output probably via an immunological mechanism; however, this cannot be conclusively proven.

**REFERENCES**


FISHER, J. M. AND TAYLOR, K. B. Unpublished data.


