Clin. exp. Immunol. (1980) **41**, 264–270.

Inhibition of parietal cell function by human gammaglobulin containing gastric parietal cell antibodies

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(Accepted for publication 22 February 1980)

SUMMARY

Parietal cell antibodies (PCA) are found in up to 90% of sera from pernicious anaemia patients but it is often stated that they could represent an epiphenomenon without being directly responsible for the achlorhydria. In the present studies a direct effect of these antibodies on the secretory function of gastric acid-secreting cells has been demonstrated in two different experimental systems. In one set of experiments IgGs containing PCA activity were shown to inhibit acid secretion specifically in the living gastric mucosa of the bull frog suspended as a diaphragm between two chambers. The other system demonstrated their inhibition of carbonic anhydrase activity in a cytochemical bioassay for human G_{17}-gastrin, suggesting a blocking effect on the gastrin receptors in the canalicular microvilli or the cell membrane. These experiments suggest a direct pathogenic role for PCA in autoimmune fundal gastritis and in pernicious anaemia.

INTRODUCTION

Autoantibodies to gastric parietal cells occur in most patients with autoimmune type A atrophic gastritis and pernicious anaemia (Strickland & Mackay, 1973; Chanarin, 1978). They are found in the serum and gastric juice, and in plasma cells present in the inflammatory response in the gastric mucosa (Strickland *et al.*, 1971). The antigen is a component of the lipoprotein membranes in the canalicular microvilli (Hoedemaeker & Ito, 1970). It is cell-specific but not species-specific. The antibodies cross-react with the gastric acid-producing cells of all mammalian species that have been tested and also with those of the bull frog, *Rana catesbiana*.

Although in the atrophic gastritis of pernicious anaemia there is frequently a severe impairment or total loss of capacity to secrete hydrochloric acid, acid production may be undetectable even when residual parietal cells are found in the gastric biopsies of these patients. Thus it is possible that parietal cell antibodies (PCA) might have an inhibitory effect on the secretory activity of otherwise functional parietal cells. In this communication we describe two separate experimental studies, using different approaches to the same problem, to investigate this possibility. The first involves a direct study of acid secretion; the second is concerned with the response of parietal cell carbonic anhydrase activity which is implicated in the process of gastric acid secretion (Davenport, 1962).

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0099-9104/80/0800-0264$02.00

MATERIALS AND METHODS

Sera were obtained from healthy subjects and from patients with pernicious anaemia. In the first study, the presence of PCA in the thirteen sera selected was demonstrated by immunofluorescence on healthy human gastric mucosa as well as a significant complement-fixation titre against gastric homogenate. Three of these also contained Type I antibodies to intrinsic factor (Ashworth *et al.*, 1967). No PCA were found by immunofluorescence in the control sera. An immunoglobulin fraction was prepared from each serum by separation on DEAE-cellulose, with Tris–phosphate buffer (Peterson & Chiazze, 1962). Each was dialysed for 24 hr against two changes of the medium used in the non-secretory chamber (see below). For the second study, four unfractionated sera, two from normal subjects and two from patients with pernicious anaemia, were tested under code. Subsequently, partially purified parietal cell or intrinsic factor antibodies were obtained by precipitation of immunoglobulins with 40% ammonium sulphate from appropriate pernicious anaemia sera containing only one or other of the antibodies, as determined by immunofluorescence or by the charcoal-binding radioimmunoassay (Ardeman & Chanarin, 1963) for intrinsic factor antibodies. Two samples of each type were examined.

Measurement of acid secretion

Acid secretion was measured in halves of the gastric mucosa of the bull frog *in vitro* by the technique of Davies (1951) modified by Harris & Edelman (1960) and by Rehm & LeFevre (1965) as shown in Fig. 1. It was necessary to use frog stomach in these experiments as mammalian gastric mucosa does not survive sufficiently well to secrete acid under these conditions. The frog stomach contains only one type of mucosal cell which secretes acid, intrinsic factor and pepsinogen. These cells cross-react well with human PCA in the indirect immunofluorescence test as seen in Fig. 2.

The stomach was removed from pithed bull frogs (about 400 g in weight), divided into two equal parts along the greater and lesser curvatures, and the muscular layer was carefully separated from the mucosa. Each half of the total, intact mucosa was then mounted as a diaphragm between two glass chambers so that one-half of each stomach could be used as a control for the other. The effective area of each mucosal 'diaphragm' was 3·8 cm^2; the volume of medium in each compartment of the double chamber was 5 ml. On the non-secretory side, the composition of the medium (mmol/l) was: NaCl (84·6), $NaHCO_3$ (17·8), KCl (3·2), $CaCl_2$ (1·8), KH_2PO_4 (0·8), $MgSO_4$ (0·8), glucose (12). On the secretory side, the bicarbonate and KH_2PO_4 were omitted, sodium chloride being substituted to achieve the same total molarity. The solution on the non-secretory side was bubbled continuously with a mixture of 95% oxygen:5% carbon dioxide and on the secretory mucosal side with 100% oxygen. The pH on the secretory side was maintained at pH 5·5 by continuous addition of sodium hydroxide (8 mmol/l) by means of a Combitrator 3D (Metrohm, Herisau, Switzerland), the rate of addition providing a measure of the rate of secretion of gastric acid. All experiments were done at room temperature.

In each experiment, the two halves of the same stomach were set up in separate pairs of chambers and allowed to attain a steady rate of spontaneous acid secretion, which was achieved in

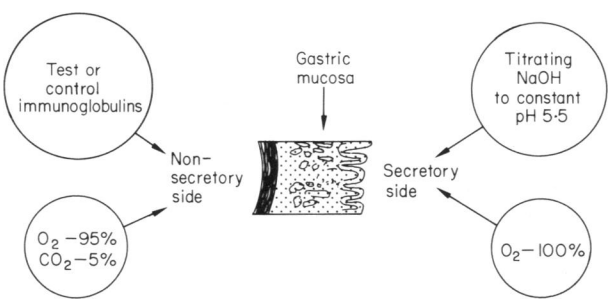

Fig. 1. Diagrammatic representation of the system used for testing gastric acid secretion in the bull frog stomach.

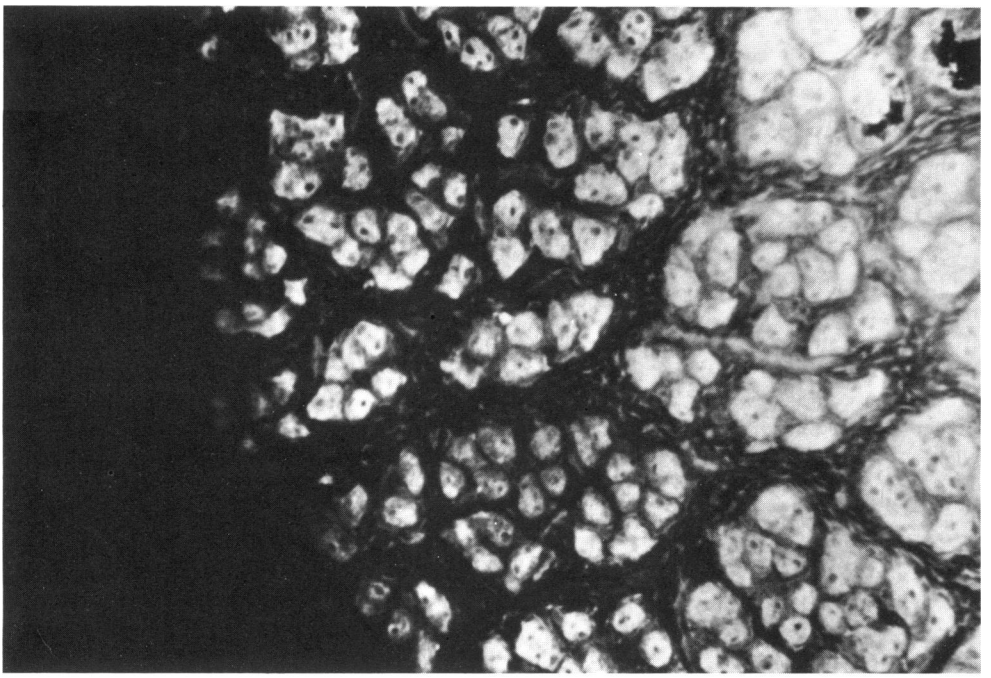

Fig. 2. Immunofluorescence of a section of bull frog stomach reacted with a serum containing gastric parietal cell antibodies (from a patient with pernicious anaemia). Sections exposed to normal serum showed only weak fluorescence throughout the section.

2–3 hr. To the non-secretory halves of each chamber, an immunoglobulin preparation was added to a final concentration of 7·5 mg/ml. Preliminary experiments were also made with saline in place of immunoglobulins.

Analysis of effect on carbonic anhydrase activity

For each experiment, strips (5 × 3 mm) of the fundus of the stomach of a single guinea-pig were maintained *in vitro* for 5 hr either in the presence or absence of the sera or immunoglobulins, as in the procedure of Loveridge *et al.* (1974). They were subsequently exposed to at least two concentrations of human synthetic G_{17}-gastrin (National Institute for Biological Standards and Control) for the previously determined optimal time of 5 min. The response was stopped by chilling the tissue to −70°C; the effect on carbonic anhydrase activity was measured by microdensitometry of the cytochemical reaction (Loveridge, 1978) in the parietal cells.

RESULTS

Acid secretion: bull frog experiments

In preliminary experiments, the spontaneous secretion of acid, 2–3 hr after removal of the frog stomach (initial rate), was 2 μEq (1·4–2·6) HCl per 15 min or 0·53 μEq HCl per cm^2 of gastric mucosa. The two halves of the same stomach rarely secreted acid spontaneously at the same rate, the most likely explanation for this being non-homogeneous distribution of gastric mucosal cells.

In a series of ten studies, with control immunoglobulin in the 'non-secretory' chamber of each pair, it was established that the half-stomach that secreted more acid initially continued to do so and that no significant change in the differences between the secretory rates of the two halves occurred over the course of nine 15-min periods. This is shown graphically in Fig. 3 (upper graph), in which

the line expressing the mean (± s.e.) of the differences for each 15-min period between the faster and slower secreting halves does not deviate significantly from the horizontal.

On the graph the mean difference between the faster and slower secreting halves in the first 15-min period, which was 0·23 μEq, has been corrected to zero, the same correction being applied to the mean for each subsequent 15-min period. This provides an easier comparison with the results depicted in Fig. 3 (lower graph).

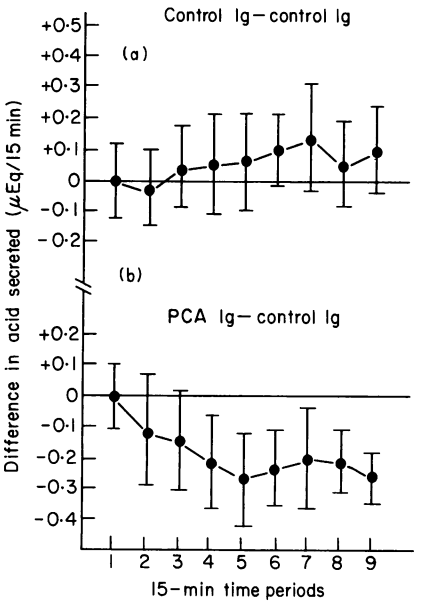

Fig. 3. Means of differences (± s.e.) in rates of acid secretion between two halves of the same stomachs. (a) Each treated with normal human IgG (ten observations). Differences in secretory rates are not significant. (b) One-half of each stomach treated with parietal canalicular antibody-containing human IgG (PCA-Ig), the other half with normal human IgG (thirteen observations). There is a progressive, significant ($P < 0{\cdot}001$) reduction in acid secretion by the PCA-Ig-treated halves.

These results provided a basis for comparison with the differences in the rates of acid secretion which occurred when preparations of control immunoglobulins or saline were added to one half, and of PCA-containing immunoglobulins to the other half, of the same stomach. This was done randomly in respect of which half was secreting acid at a higher rate initially. The results are shown graphically in the lower part of Fig. 3. The mean difference in initial rates in thirteen experiments was zero. The mean value of the differences increased with time throughout the course of the experiments and in the second hour it was 14% compared with initial values. Analysis of the results using the statistical method of Hills (1968) for data derived from sequential experimental observations indicates that the depression of acid secretion in the presence of PCA-containing Igs, compared with control immunoglobulins or saline, was significant at a level of $P < 0{\cdot}001$.

Carbonic anhydrase activity: cytochemical bioassay experiments

In the first experiments, each of the four coded sera were added to the strips of guinea-pig fundus, after the 5-hr maintenance period, at dilutions of $1:10^2$ and $1:10^3$ (in T8 culture medium). Four other mucosal strips were treated with one of a graded series of concentrations (0·005–5·0 pg/ml) of human G_{17}-gastrin. The dose–response produced by two of the sera was parallel to that caused by the standard G_{17}-gastrin; that of the other two sera was decidedly not parallel, suggesting that they contained a factor that interfered with the activity of the gastrin. Recovery of G_{17}-gastrin activity added to the normal sera (5 and 500 pg/ml respectively) was complete; there was no recovery of

exogenously added gastrin activity in the two PA sera until the samples had been diluted at least $1:10^4$. When normal sera were included in the medium for the 5-hr maintenance culture at $1:10^2$ dilution and the fundic strips tested for the stimulation of carbonic anhydrase activity by exposure to G_{17}-gastrin (5 and 0·005 pg/ml) in the absence of these samples, the stimulation was virtually the same (9 and 8% depressed, respectively) as that found in control strips which had received no serum during the maintenance period. On the other hand, when a pernicious anaemia serum was added to the medium for the 5-hr maintenance period, the stimulation of carbonic anhydrase activity was inhibited by 90% when the strips were subsequently exposed to the same concentrations of gastrin.

In the second set of bioassays, the immunoglobulin fractions containing either PCA or intrinsic factor antibodies were reconstituted to the original volume of serum in Coombs' buffer. Strips of gastric fundus were maintained for 5 hr in the normal Trowell's T8 medium containing one or other of these antibodies at a dilution of $1:10^2$. They were then subjected to fresh medium containing G_{17}-gastrin at either 5·0 or 0·005 pg/ml in the absence of these antibodies, and the carbonic anhydrase activity was measured. The effect of these two antibodies on the carbonic anhydrase response to gastrin is shown in Fig. 4. The presence of intrinsic factor antibodies in the maintenance

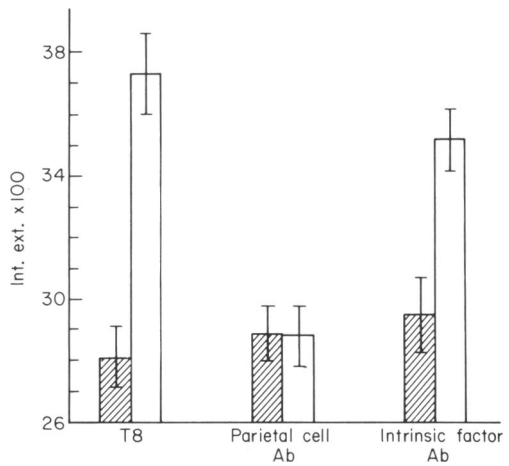

Fig. 4. The effect of exposure to PCA and intrinsic factor antibodies on the subsequent response of parietal cells to gastrin. Strips of guinea-pig fundus, maintained for 5 hr in the presence of the normal medium (T8) with or without either PCA or intrinsic factor antibodies, were then exposed to G_{17}-gastrin, at 0·005 (▨) or at 5·0 pg/ml (□). The stimulation of carbonic anhydrase activity per parietal cell (integrated extinction of the reaction product: Int. ext. × 100), seen in the cells exposed only to the T8 medium, was abolished in those exposed previously to PCA but was largely unaffected in those treated with intrinsic factor antibodies. Bars indicate the standard error of the mean of at least twenty readings.

culture affected the subsequent response to gastrin only slightly (24 and 38% inhibition in two studies). In contrast, PCA caused marked inhibition of the subsequent ability of the parietal cells to respond to gastrin (82 and 99% inhibition in two studies).

DISCUSSION

It is well known that there is a strong association between the presence of circulating parietal cell antibodies and inflammation and atrophy of the acid-secreting gastric mucosa. This is most obvious in pernicious anaemia in which there is either severe hypochlorhydria or achlorhydria. Because gastric acid secretion is sometimes undetectable even when some parietal cells can still be found in gastric biopsies, it was considered possible that these antibodies might impair or inhibit parietal cell function.

Previous work by Glass and his associates (Glass, 1977) had shown that repeated injections of human PCA into rats *in vivo* led to a reduction in acid secretion after 6–8 weeks whereas similar injections of intrinsic factor antibodies only reduced the pepsin and intrinsic factor output. In further experiments these authors showed that the effect of the PCA was not due to direct cytotoxicity but to an inhibition in the maturation or proliferation of the gastric cells (Lopes, Ito & Glass 1976). Thus the aim of the present studies was to test the direct effect of gastric antibodies on the function of these cells *in vitro*. This has been investigated in two different experiment systems.

It was first shown that the immunoglobulin fraction from sera of patients with pernicious anaemia directly inhibited gastric acid secretion. This was found with all thirteen sera, of which only three had antibodies to intrinsic factor; all contained PCA. An approach to the mechanism of this inhibition was followed by analysing the influence of other samples of serum on parietal cell carbonic anhydrase activity. Two pernicious anaemia sera gave non-parallel dose–responses when tested, against a standard preparation of gastrin, for their ability to stimulate carbonic anhydrase activity in living gastric parietal cells *in vitro*. Thus if they contained gastrin, it was not allowed to act in an analogous way to the standard preparation in Trowell's medium. Moreover the same applied to gastrin added to these sera, although this was active when the serum was diluted $1:10^4$. These findings implied the presence in these sera (but not in the sera from normal subjects) of some factor that inhibited the expression of gastrin, either by reacting with the hormone or with the gastrin receptor on the parietal cells (Soumarmon, Cheret & Lewin, 1977). To determine between these possibilities, the cells were exposed to the sera during the 5-hr maintenance culture; the sera were then removed and the responsiveness of the parietal cells to gastrin was tested. Under these conditions, the lack of response in cells exposed previously to the pernicious anaemia serum indicated the presence in this serum of a factor that binds to the cells and inhibits their subsequent ability to respond to gastrin. The results with immunoglobulin fractions, containing either PCA or intrinsic factor antibodies, confirmed that of these two, only parietal cell antibodies extracted from pernicious anaemia serum can act in this way. Thus the combined study has shown that parietal cell antibodies inhibit gastric acid secretion and interfere with the associated stimulation of carbonic anhydrase activity in the parietal cells, apparently by binding to the gastrin receptor. Although a direct cytotoxic action of PCA on the gastric parietal cells has not been demonstrated owing to difficulty in keeping the isolated cells alive, the fact that PCA interfere with the ability of these cells to respond to gastrin, as measured in these studies, suggests that the trophic action of the hormone might also be blocked. Such a blockade might stop the proliferation and maturation of parietal cells, required to replace loss of older cells in the turnover of the tissue, so leading to mucosal atrophy.

Part of this study was supported by USPHS Grant AMO6971 and United States Veterans Administration. Work in the Department of Immunology is supported by the WHO and the Medical Research Council.

Dr T. U. Hausamen was a post-doctoral Fellow of the German Academic Exchange Service; Dr J. M. Fisher was a Dernham Fellow, California Division of the American Cancer Society.

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