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Gastric parietal cell antibodies: demonstration by immunofluorescence of their reactivity with the surface of the gastric parietal cells

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SUMMARY

Viable, intact gastric cells were obtained by pronase digestion of inverted rat stomach. The cell suspensions contained two main distinct cell populations, i.e. 'large' cells (mean diameter 16 μm) and 'small' cells (mean diameter 8·5 μm). By indirect immunofluorescence on smears of dispersed rat gastric cells, the large cells were identified as parietal cells, since all the sera containing parietal cell antibodies (PCA) were seen to react with the cytoplasm of these cells, leaving the cytoplasm of the small cells completely unstained. Thirty-one PCA-positive sera and forty-one PCA-negative sera were tested for gastric cell surface-reactive antibodies by an indirect immunofluorescence technique on suspensions of viable gastric cells. All the PCA-containing sera yielded a membrane immunofluorescence confined to the large cells, while none of the PCA-negative sera induced this fluorescent pattern. The surface reaction persisted unmodified when $F(ab')_2$ fragments processed from IgG PCA-positive sera and FITC-conjugated pepsin fragments of rabbit IgG directed against the $F(ab')_2$ fragments of human IgG were employed for the membrane fluorescence studies. The absorption of PCA-positive sera with viable, intact gastric cells led to the disappearance of both the surface immunofluorescence of the viable large cells and the cytoplasmic fluorescence of the rat parietal cells. These results demonstrate that PCA invariably react with an antigen represented on the surface of parietal cells, and that this antigen is immunologically identical to the intracytoplasmic 'microsomal' antigen.

INTRODUCTION

Complement-fixing antibodies reacting with homogenates of gastric mucosa were first demonstrated in pernicious anaemia sera by Irvine *et al.* (1962) and by Markson & Moore (1962). These antibodies are best detected by indirect immunofluorescence on cryostat sections of human or rat stomach (Taylor *et al.*, 1962; Irvine, 1963; Doniach & Roitt, 1964), and are associated with a type of chronic gastritis which affects mainly the fundal mucosa, usually sparing the antrum—type A gastritis, according to Strickland & Mackay (1973).

When indirect immunofluorescence on cryostat sections of stomach is used, the gastric parietal cell antibodies (PCA) are seen to stain diffusely the cytoplasm of the parietal (oxyntic) cells. Ultracentrifugation studies of gastric mucosal homogenates showed that the parietal antigen is mostly localized to the 'microsomal' fraction (Baur, Roitt & Doniach, 1965), and detailed bio-

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chemical analysis revealed that it shares many characteristics with the microsomal antigen of thyroid cells: the two antigens are both inactivated by surface-active detergents, but neither is affected by ultrasonic disintegration or by treatment with phospholipase, ribonuclease or dilute alkali (Roitt & Doniach, 1976). The work by Ward & Nairn (1972) provides good evidence that the parietal cell antigen is a lipoprotein associated with smooth cytoplasmic membranes, and the ultrastructural study made by Hoedemaeker & Ito (1970) with peroxidase-coupled PCA indicates that the antigen is localized to the microvillus membranes of the intracellular canalicular system of the parietal cells. In a short abstract, Zeitoun, Voillemot & Lewin (1975) mentioned the existence of surface parietal cell antigen(s) detectable by immunoelectron microscopy with peroxidase-labelled antisera, but complete evidence for this is still lacking. Antibodies to plasma and nuclear membranes of guinea-pig gastric parietal cells were experimentally raised in rabbits, and the failure of absorption of these antibodies with an excess of gastric microsomes led Jewell *et al.* (1975) to postulate that the parietal cells possess membrane antigen(s) distinct from the microsomal antigen.

The recent development of satisfactory techniques for obtaining viable and isolated cells from the rat gastric mucosa prompted us to search for gastric cell membrane-reactive autoantibodies.

MATERIALS AND METHODS

Isolation and separation of gastric cells

Wistar strain rats weighing 250 to 300 g were used throughout these experiments. Rats were killed and their stomach was removed and inverted as described by Dikstein & Sulman (1965). To obtain dispersed gastric cells, the pronase method reported by Lewin *et al.* (1974) and by Soumarmon, Cheret & Lewin (1977) was employed with slight modifications. Briefly, the pronase enzyme (Merck, 70,000 u/g) was dissolved in pH 7·4 buffered Krebs-type medium (NaH_2PO_4 0·5 mM; Na_2HPO_4 1 mM; $NaHCO_3$ 20 mM; NaCl 70 mM; KCl 5 mM; HEPES 50 mM; glucose 80 mM; BSA 2%; EDTA 2 mM) to obtain a final concentration of 1,000 u/ml. Three millilitres of the dissolved enzyme was injected through a fine-gauge canula into the sac formed by the inverted stomach, which was then soaked into three consecutive 30-min baths containing Krebs-type medium kept at 37°C. The digestion in the last bath was completed by delicate magnetic agitation at room temperature for 30 min. The residual stomach was then removed, and the crude cell suspensions were filtered through a 20-ml plastic syringe, filled to 1·5 ml with siliconized glass beds, 2 mm in diameter, and equipped, instead of the needle, with a small plastic cone containing a siliconized metal filter (3 mm in diameter, 75 mesh, bar 50 μm, hole 283 × 283 μm; E. M. Specimen Grids, Beckman, Geneva). Cell separation with progressive enrichment for large cells (i.e. parietal cells) was obtained by repeated 45-sec centrifugations at 740 r.p.m. in Krebs-type medium, as reported by Soumarmon *et al.* (1977). After five centrifugation steps, the cell pellet was resuspended in a few millilitres (2 to 5 ml according to the mass of the pellet) of Krebs-type medium. The viability of the isolated gastric cells was assessed by the classical vital dye (trypan blue and eosin) exclusion test. Smears of dispersed gastric cells were stained with haematoxylin and eosin or with the May–Grünwald–Giemsa stain.

Human sera

All the sera used in the present experiments were previously tested by classical indirect immunofluorescence on composite cryostat sections of rat stomach, rat liver, rat kidney and human group O thyrotoxic thyroid gland for the following autoantibodies: gastric parietal cell antibodies (PCA), thyroid microsomal antibodies (TMA), smooth muscle antibodies (SMA), mitochondrial antibodies (MA) and reticulin antibodies (RA). Sera producing weak staining (+) of the rat parietal cells were excluded, while sera giving rise to a staining of the rat parietal cells and to a simultaneous staining of other rat tissues, such as kidney (brush border of the proximal tubules) and liver (Küppfer cells), were retested on cryostat sections of human stomach, surgically removed from a group O patient with a duodenal ulcer. This allowed us to avoid false-positive reactions due to heterophile antibodies, which react with xenospecific and allospecific antigens, but are not tissue-specific (Hawkins, McDonald & Dawkins, 1977). The precise autoantibody content of the sera is shown in Table 1.

Twenty-nine PCA-positive sera and thirty-eight PCA-negative sera were employed for the tests on smears of isolated gastric cells. Thirty-one PCA-positive sera (eleven of which were positive also when tested on smears of isolated gastric cells) and forty-one PCA-negative sera were tested for membrane immunofluorescence on viable gastric cells.

Most of the PCA-containing sera were obtained from patients with histologically proven atrophic gastritis and/or with vitiligo.

All sera were kept frozen at −20°C until use, and were diluted 1:5 in saline before testing. The sera employed for surface immunofluorescence experiments were filtered through 0·45-μm Millipore discs and inactivated at 56°C for 30 min, to avoid possible cytotoxic effects.

Fractionation of IgG PCA-positive sera

Two sera containing high titres of PCA belonging to the IgG class were pooled and the IgG fraction was isolated by DEAE-cellulose chromatography (Whatman DE-52) with 0·015 M phosphate buffer, pH 7·6 (Michaelsen & Natvig, 1972). The $F(ab')_2$ fragments of IgG were then obtained by the classical method of pepsin digestion, according to Nisonoff *et al.* (1960).

Fluorescein-conjugated antisera

All screening tests for autoantibodies on cryostat tissue sections were performed using FITC-conjugated goat antiserum to human gammaglobulins (Menarini Reagents, Italy). About half of the sera were also tested by using monospecific anti-human IgG conjugates produced in rabbits (DAKO Immunoglobulins).

The same lots of monospecific conjugates were employed for the tests on smears of dispersed gastric cells, as well as for the surface immunofluorescence experiments. Parallel tests for membrane immunofluorescence were performed using two additional FITC-conjugated antisera specific for the $F(ab')_2$ fragment of human IgG, namely a commercial goat IgG preparation (Cappel Laboratories, Cochranville, Pennsylvania, USA) and an antiserum raised in rabbits. This latter antiserum was prepared by one of us (F.S.) in Dr Natvig's Laboratory (Institute of Immunology and Rheumatology, Oslo, Norway) as reported elsewhere (Frøland & Natvig, 1972), and the pepsin fragment of the rabbit IgG directed against the $F(ab')_2$ fragments of human IgG was obtained according to Nisonoff *et al.* (1960), with minor modifications.

The optimal dilution of the conjugates was previously assessed by the results obtained with two standard PCA-containing sera from our laboratory. The commercial goat antiserum to human $F(ab')_2$ fragments was used at a dilution of 1:5, and the Fab fragment specific antiserum prepared in Dr Natvig's laboratory was employed at a dilution of 1:16.

Indirect immunofluorescence on smears of isolated gastric cells

Smears of isolated gastric cells were obtained by gently distributing one drop of gastric cell suspension over the surface of a microscope slide. The smears were then air-dried, washed with PBS, pH 7·4, for 10 min at room temperature and used either unfixed or fixed for 30 min at room temperature in alcohol–ether (60 vol/40 vol). The classical indirect immunofluorescence test was performed on freshly prepared smears or on smears stored for 12 to 36 hr at −80°C.

Surface immunofluorescence on viable gastric cells

Incubation of 0·2 ml of a gastric cell suspension was performed in centrifuge tubes with an equal volume of the test serum for 30 min at room temperature. After incubation, the cells were washed three times at 4°C by suspension in 5 to 8 ml of Krebs-type medium, followed by centrifugation at 1,200 r.p.m. for 5 min. The cells were then incubated at room temperature for 30 min with 0·2 ml of conjugated antiserum, washed again three times at 4°C and resuspended in 0·2 ml of Krebs-type liquid. One drop of the cell suspension was placed on a slide with a coverslip, and the preparation was examined immediately for surface immunofluorescence by means of a Leitz Ortholux fluorescent microscope, equipped with a Ploem vertical illuminator. Cell viability was controlled throughout the study. Photographs were taken with a Leitz Orthomat apparatus, using Kodak Ektachrome high-speed 400 ASA films or Ilford HP5 400 ASA films. Controls included incubation for 30 min at room temperature of gastric cell suspensions with serum alone and with conjugates alone.

Absorption experiments

Five PCA-containing sera were pooled, heat-inactivated and filtered through 0·45-μm Millipore filters. The autoantibody titre of the pool, as assessed by indirect immunofluorescence on cryostat section of rat stomach, was between 1:32 and 1:64.

Dispersed gastric cells were derived from ten stomachs of rats killed simultaneously, and, through repeated centrifugations, preparations containing more than 75% of large cells were obtained, as estimated under light microscopy.

Three sets of absorption experiments were made with gastric cells. In the first series of experiments, 0·3 ml of a pooled serum diluted 1:2 was incubated for 60 min at room temperature, and for an additional 60 min at 4°C with a gastric cell preparation containing an absolute number of 'large' gastric cells equal to 5×10^6. In the second series of experiments, 0·3 ml of a pooled sample diluted 1:5 was held as above with a gastric cell preparation containing 4×10^7 large cells. In the third series of experiments, 0·3 ml of a pooled serum diluted 1:16 was incubated for 60 min at room temperature with an equal volume of gastric cell pellet enriched in large cells.

In all the absorption experiments, cell viability was estimated by the trypan blue exclusion test at the beginning and at the end of the incubation time. The absorbed serum samples were immediately tested for surface immunofluorescence with freshly prepared rat gastric cell suspensions and for cytoplasmic fluorescence of parietal cells on cryostat sections of rat stomach. Parallel tests were carried out with the unadsorbed and appropriately diluted pooled serum. The results of the fluorescence tests were read independently by three observers, and the intensity of the fluorescence was arbitrarily graded from 0 to + + + +.

In other absorption experiments, 0·3 ml of a pooled serum diluted 1:5 was incubated for 60 min at room temperature with an equal volume of packed human group AD+ erythrocytes or with 20 mg of rat liver powder.

RESULTS

Separation and identification of gastric cells

Pronase digestion has proved to be a suitable method for separating cells from intact rat gastric mucosa. In the present experiments, the purpose of which was to test the possible existence of gastric cell surface-reactive antibodies, little importance was attached to the absolute number of cells derived from each rat stomach, while the greatest attention was directed towards obtaining viable, intact and well washed gastric cells. Indeed, the isolated gastric cells we obtained had a high degree of viability, as assessed by the supravital dye exclusion test, whereas up to 92% of the separated cells excluded trypan blue and eosin within 90–120 min of preparation.

The initial (uncentrifuged) crude suspension contained isolated cells whose diameter ranged from approximately 6·5 to 20 μm. Two extreme types of cells were readily identifiable under light microscopy by their diameter, namely 'large' cells (diameter > 12 μm, mean diameter 16 μm) and 'small' cells (diameter < 10 μm, mean diameter 8·5 μm). For simplicity, we will refer to these extreme cell populations, although in our studies we have seen that some intermediate-sized gastric cells also had the morphological and the immunological characteristics of the large cells.

The large cells represented 20–25% of the entire cell population. After repeated centrifugations, the percentage of large cells rose to 70–80%.

All of the large cells stained typically with haematoxylin and eosin, and fulfilled the morphological characteristics which led Walder (1968) to consider them as parietal cells (Fig. 1a). This interpretation was confirmed by our immunofluorescence studies on smears of dispersed gastric cells: all the PCA-positive sera on cryostat sections of rat stomach yielded a brilliant cytoplasmic fluorescence of the large cells, leaving the cytoplasm of the small cells completely unstained (Fig. 1b); conversely, none of the PCA-negative sera reacted with the large cell cytoplasm (Table 1).

Sera containing mitochondrial antibodies stained the large cells in a fashion similar to the PCA-containing sera, while sera containing other autoantibodies did not induce any cytoplasmic fluorescence.

Of the total of sixty-seven sera tested on smears of gastric cells, two PCA-negative sera were seen

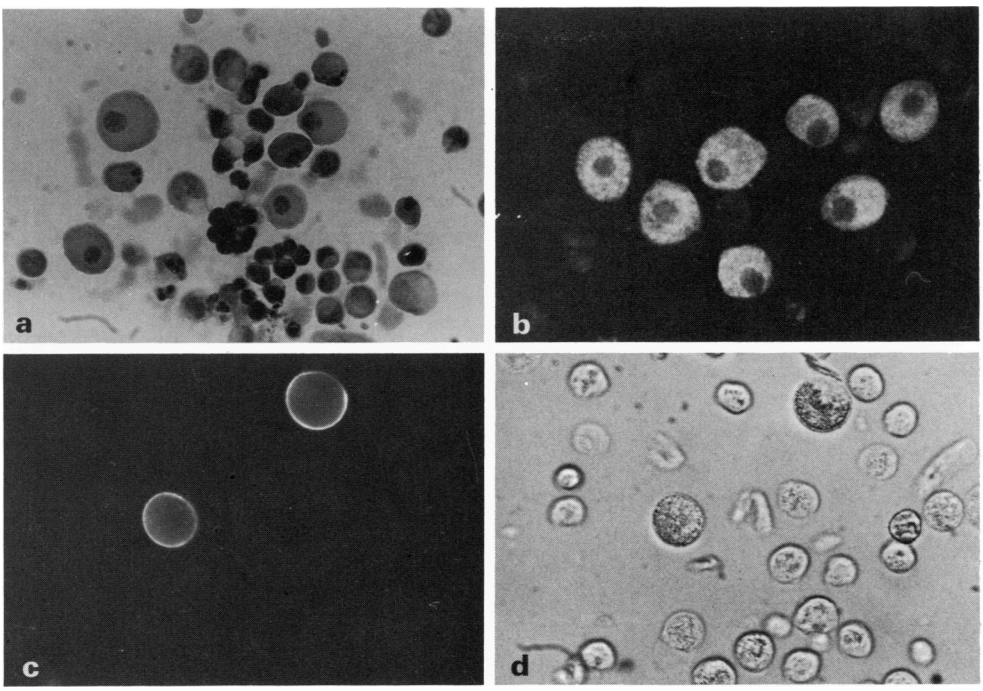

Fig. 1. (a) Haematoxylin & eosin staining of a smear of dispersed rat gastric cells. Two main populations are easily distinguishable by their diameter (i.e. large cells and small cells) and by their tintorial characteristics. (b) Smear of isolated rat gastric cells stained by indirect immunofluorescence with a serum containing parietal cell antibodies (PCA): a strong cytoplasmic fluorescence is displayed exclusively by the large cells, while the other hardly visible fluorescent 'shadows' correspond to the small cells. (c) Indirect immunofluorescence on viable rat gastric cells in suspension, incubated with a PCA-containing serum: two cells display a bright membrane immunofluorescence. The observation of the same field under light microscopy (d) reveals that these two cells are both large cells, and that in the field were present numerous 'other' cells, none of which had displayed surface immunofluorescence. (For all illustrations, original magnification × 400.)

to induce an unusual pattern, namely a strong cytoplasmic fluorescence in a few scattered small cells: these sera came from one apparently healthy subject without circulating autoantibodies and from one ANA-positive patient with chronic liver disease (Table 1).

Surface immunofluorescence

An absolute correlation was found between cytoplasmic fluorescence of the parietal cells and surface immunofluorescence of the large gastric cells. In fact, all the PCA-containing sera, as assessed by tests carried out on cryostat sections of rat stomach and, for eleven sera, on smears of dispersed gastric cells, gave a membrane fluorescence of the large gastric cells in suspension, whereas none of the PCA-negative sera induced cell surface immunofluorescence, with the sole exception of a few scattered spots on occasional cells (Table 1).

The specificity of the reaction for the surface of the large cells was absolute, since none of the thirty-one PCA-positive sera stained the surface of the small cells. Conversely, four PCA-negative sera were seen to induce a membrane fluorescence confined to a few small cells (Table 1).

PCA-containing sera usually induced a continuous and brilliant fluorescent ring strictly confined to the surface of the large cells, as assessed also by the sequential observation of fields under light microscopy (Fig. 1c, d). In general, the brightness of the membrane fluorescence was proportional to the PCA titre. Less frequently, the large cells, or some of them, displayed different patterns of membrane fluorescence, such as bright 'spikes' uniformly distributed over the cell surface or separate clusters of fluorescent grains ('patchy' reaction); occasionally, a 'cap-like' appearance was

Table 1. Indirect immunofluorescence on smears of isolated gastric cells and on viable rat gastric cells in suspension

Sera (autoantibody content)*	IFL on gastric cell smears				IFL on viable gastric cells			
	No. of sera tested	No. +ve for 'large' cell cytoplasm	No. +ve for 'small' cell cytoplasm	No. +ve for nuclei	No. of sera tested	No. +ve for 'large' cell surface	No. +ve for 'small' cell surface	No. +ve for nuclei
PCA +ve, other AAbs −ve	20	20	0	0	22	22	0	0
PCA +ve, TMA +ve	5	5	0	0	5	5	0	0
PCA +ve, ANA +ve	3	3	0	3	4	4	0	0†
PCA +ve, SMA +ve	1	1	0	0	—	—	—	—
PCA −ve, other AAbs −ve	31	0	1	0	23	0	4	0
PCA −ve, TMA +ve	1	0	0	0	10	0	0	0
PCA −ve, ANA +ve	3	0	1	3	3	0	0	0†
PCA −ve, 'M' A +ve	3	3	0	0	3	0	0	0
PCA −ve, HA +ve	—	—	—	—	2	0	0	0

* As assessed by indirect immunofluorescence on cryostat tissue sections.

† A weak nuclear staining was observed only in the few non-viable, disrupted cells.

IFL = indirect immunofluorescence; AAbs = autoantibodies; PCA = gastric parietal cell antibodies; TMA = thyroid microsomal antibodies; ANA = antinuclear antibodies; SMA = smooth muscle antibodies; 'M' A = mitochondrial antibodies; HA = heterophile antibodies, which induced a cytoplasmic staining of the rat parietal cells.

observed in a very limited number of cells. The few non-viable cells present in the preparation were easily distinguishable from living, intact cells, since they displayed a strong, sometimes irregular, diffuse fluorescence, were usually markedly altered in shape, and had became permeable to trypan blue. None of the PCA-negative sera that contained thyroid microsomal autoantibodies or other antibodies, including heterophile antibodies, gave a surface immunofluorescence of the large cells (Table 1).

The tests done by incubating gastric cell suspensions with $F(ab')_2$ fragments derived from IgG PCA-positive sera, followed by incubation with conjugated pepsin fragments of rabbit IgG specific for the $F(ab')_2$ fragments of human IgG, gave rise to a surface immunofluorescence of the large cells which was indistinguishable from that induced by non-pepsin-treated sera and antisera.

The different conjugates employed for the surface experiments all induced membrane immunofluorescence of the large cells in the presence of PCA. However, the best results were obtained by using the $F(ab')_2$ specific antiserum produced in rabbits: this antiserum yielded a neat and bright surface fluorescence, with a minimal fluorescent halo and without any appreciable non-specific fluorescence of the small cells.

The control experiments invariably gave negative results. No autofluorescence was displayed by the viable gastric cells.

Absorption experiments

The absorption of PCA-containing pooled sera with rat liver powder or with human group AD+ erythrocytes did not induce any significant change in membrane immunofluorescence on viable gastric cells, nor in cytoplasmic fluorescence of the parietal cells. The absorption of the test sample with 5×10^6 large gastric cells led to an appreciable decrease (from $+++$ to $++$) of cytoplasmic fluorescence of the rat gastric cells and to a doubtful change of surface immunofluorescence. However, the absorption of the pooled serum with 4×10^7 large cells, or with an equal volume of a packed gastric cell preparation enriched in large cells, led to the complete disappearance of both surface immunofluorescence of the large cells and cytoplasmic fluorescence of the parietal cells on cryostat sections of rat stomach.

Up to 95% of the absorbing gastric cells were viable at the beginning of the incubation time, and this percentage was practically unmodified after 1 hr of incubation and only slightly reduced after 2 hr of incubation (85–90% of viable cells). However, almost all the cells which apparently had lost their viability during the incubation time, still retained their morphological appearance intact, and only 1–2% of them displayed changes in shape and/or signs of disruption.

DISCUSSION

During the last few years, a number of proteolytic enzymes have been used to obtain dispersed gastric cells, including trypsin, papain, collagenase, pancreatin and pronase (see Haffen, Lewin & Robberecht, 1979). Pronase digestion, first developed by Blum *et al.* (1971), has proved to be one of the most effective methods for dissociating cells from the intact gastric mucosa of rats, mice, guinea-pigs and rabbits (Ito, Munro & Schofield, 1977).

Pronase digestion of *in vitro*-inverted rat stomach preparations (Lewin *et al.*, 1974; Soumarmon *et al.*, 1977) leads to suspensions containing more than 90% of viable, intact cells, well isolated from each other, and easily distinguishable by their diameter into two main types, namely 'large' cells and 'small' cells. Early morphological studies (Walder, 1968; Jewell *et al.*, 1975) and cytochemical analyses (Lewin *et al.*, 1974) provided strong evidence that the large cells represented the parietal cells, and the first step in this work, done by indirect immunofluorescence on smears of isolated gastric cells, demonstrates that only the large cells possess the parietal antigen.

In our studies on smears of dispersed gastric cells, an unusual pattern of fluorescence was given by two PCA-negative sera, which stained selectively the cytoplasm of few scattered small cells. In addition, four PCA-negative sera were seen to induce a membrane fluorescence confined to a few small cells. Studies are now in progress to identify these cells. The recent work by Vandelli *et al.* (1979) shows that a small proportion of PCA-negative patients with antral (type B) gastritis have an

autoantibody reacting with the gastrin-producing cells, thus demonstrating that gastric autoantibodies other than PCA and intrinsic factor antibodies do exist.

The present work clearly demonstrates that PCA invariably react with an antigen represented on the surface of the large cells from rat stomach. The antibody nature of this membrane reaction was confirmed by its persistence when pepsin-treated sera and antisera were used for the surface immunofluorescent tests. No attempts were made to characterize the surface antigen. However, the absolute correlation between 'microsomal' and plasma membrane reactivity strongly suggests that the surface antigen of the large gastric cells is similar, if not identical, to the microsomal antigen of the parietal cells. The disappearance of both surface and cytoplasmic fluorescence after absorption of PCA-positive sera with intact, viable gastric cells further supports this view. The sharing of antigen(s) between cytoplasm and surface is not an entirely surprising phenomenon, in view of the current concepts on plasma membranes and of the demonstration by Ito *et al.* (1977) that the surface membrane of the parietal cells may be either smooth or almost entirely covered with numerous microvilli that closely resemble the microvilli lining the intracellular canaliculi and which might be formed from the tubulovesicular membranes. These observations were made by electron microscopy and by scanning electron microscopy on isolated mouse gastric cells, but it seems that the different morphological appearances seen *in vitro* reflect, at least in part, those that an oxyntic cell *in situ* may assume in relation to its state of functional activity (Forte, Machen & Forte, 1977; Sachs, Spenney & Lewin, 1978).

In general, autoantibodies directed against cell surface antigens are expected to induce cytotoxic effects or to influence vital cellular functions. PCA are known to be capable of inducing several biological effects *in vivo* and *in vitro*. Parietal cell atrophy and suppression of acid secretion was observed in rats injected daily with IgG fractions of PCA-containing sera (Tanaka & Glass, 1970; Inada & Glass, 1975), and a similar effect was shown in dogs receiving rabbit anti-dog parietal cell antisera (Walder, 1968). Acute inflammatory changes confined to the stomach (Hausamen, Halcrow & Taylor, 1969a), and inhibition of DNA synthesis in gastric mucosa (Hausamen, Halcrow & Taylor, 1969b) were obtained by injecting guinea-pigs with rabbit antisera against antigens from foetal or adult guinea-pig gastric mucosa. Recently, it has been demonstrated that PCA are able to interfere *in vitro* with carbonic anhydrase secretion, thus inhibiting the gastrin responsiveness of the parietal cells (Bitensky *et al.*, 1979). All these well documented biological effects are difficult to explain on the basis of an autoantibody directed to an intracytoplasmic antigen and unable to enter intact and living cells, but they are much more easy to interpret if the autoantibody was to react with an antigen represented on the cell surface. Cell membrane autoantibodies could be cytotoxic for the target cells in the presence of complement and/or could mediate cytotoxicity by K (killer) cells. Furthermore, provided the microsomal antigens are somehow involved in the secretory functions of the corresponding cells, then small amounts of the antigen could be released from the cells, giving rise to the local formation of antigen–antibody complexes when specific autoantibodies are present. Some procedures have been recently developed for maintaining gastric parietal cells in culture (Munro *et al.*, 1975; Kasbekar & Blumenthal, 1977), and attempts are now being made in our laboratory to examine, in different *in vitro* systems, the possible direct cytotoxic effect of PCA or their ability to act as mediators of potentially damaging effector cells. *In vitro* studies on T cell hypersensitivity reactions against the surface autoantigen of the gastric parietal cells have also been undertaken.

Although the concept of the existence of an 'autoimmune' gastritis is now widely accepted, the mechanisms by which autoimmunization to the parietal cell antigen operate are still poorly defined. The possibility of demonstrating antigen–antibody reactions on the surface of viable gastric cells may represent the first step towards a better understanding of the pathogenesis and the pathophysiology of human autoimmune gastritis. Finally, other known microsomal autoantibodies might prove to have cell surface reactivity: the preliminary work by Khoury *et al.* (1979) provides strong evidence that the thyroid microsomal antibodies also react with an antigen represented on the surface of thyroid cells.

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