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The Immunoglobulin Class of Autoantibody-containing Cells in the Gastric Mucosa

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Summary. Autoantibody to Castle's intrinsic factor in mucosal plasma cells in gastric biopsies of patients with pernicious anaemia has been shown to be of the IgG type, using a double-labelling technique. The role of IgG immunoglobulins compared with that of IgA immunoglobulins in the immune response to autoantigens and to foreign proteins in the gastrointestinal tract is discussed.

INTRODUCTION

Autoantibodies to a microsomal component of gastric parietal cells and to Castle's intrinsic factor can be demonstrated in the serum and gastric juice of many patients with pernicious anaemia. Plasma cells containing such autoantibodies have been demonstrated in the inflamed gastric mucosa of some patients with pernicious anaemia (Baur, Fisher, Strickland and Taylor, 1968). The presence of such cells containing intrinsic factor autoantibody correlates well with that of IgG autoantibody to intrinsic factor in the serum of these patients (Strickland, Baur, Ashworth and Taylor, 1970). We have now examined the immunoglobulin class of the autoantibody in these gastric mucosal plasma cells.

MATERIALS AND METHODS

Gastric biopsies were obtained from five patients with pernicious anaemia diagnosed by the following criteria. A Schilling test showing significant impairment of vitamin B_{12} absorption which was restored by intrinsic factor, the finding of atrophic gastritis or gastric atrophy on gastric biopsy and the absence of gastric acid production in response to maximal doses of betazole hydrochloride. Peroral gastric biopsies, obtained with a multipurpose suction biopsy tube (Quinton Instrument Co., Seattle, Wash.), were preserved by the technique of Sainte-Marie (1962). Any plasma cells containing antibody to a complex of intrinsic factor and vitamin B_{12} in 6 μ sections of these gastric biopsies were identified by a modification of the radioautographic method previously described (Baur *et al.*, 1968). The method permitted the characterization of the immunoglobulin type of the antibody-containing cell by the techniques of combined immunofluorescence staining and radioautography described by McDevitt, Askonas, Humphrey, Schecter and Sela (1966). Briefly, deparaffined biopsy sections were incubated with a complex of ^{57}Cobalt-labelled vitamin B_{12} (specific activity 80–120 μCi/μg) and intrinsic factor, washed, then flooded with specific fluorescein isothiocyanate-conjugated anti-human IgG (Hoechst, Kansas

City, Mo.) or anti-IgA (Melpar Inc., Falls Church, Va.). After washing, sections were covered with Kodak AR-10 stripping film and stored at 4°. Multiple sections for each immunoglobulin type were made from each biopsy and developed at varying time intervals, then observed and photographed with a Zeiss U.V. microscope with an Osram G200 U.V. source. Using a UG5 primary excitation filter and a No. 41 barrier filter the plasma cells stained with specific immunoglobulin-class antiserum appear green. With a Kodak Wratten 25 red filter the grains in the emulsion resulting from the attachment of the complex of radioactively labelled vitamin B_{12} and intrinsic factor to cells containing antibody to intrinsic factor appear red. Using high-speed Kodak Ektachrome film a single exposure of the fluorescent cells was first made, then a double exposure showing both the fluorescence and the grains was taken. By comparing the two photographs it was possible to determine the immunoglobulin type of the cells containing antibody to intrinsic factor.

Serum and gastric juice intrinsic factor antibodies Type II were determined by the co-precipitation method of Samloff and Barnett (1965).

RESULTS

In this study we used gastric biopsies from five subjects with pernicious anaemia in which we had previously demonstrated inflammatory cells containing antibody to intrinsic factor complex by incubating gastric sections with a complex of intrinsic factor and $[^{57}CoB]_{12}$, applying stripping film, developing, and staining with Wright's stain. Under those conditions a few of the many inflammatory cells present in each section were shown to contain the autoantibody; that is, to bind the complex of vitamin B_{12} and intrinsic factor. By the present technique, cells containing the autoantibody are more difficult to recognize because only their cytoplasm is stained by monochromatic dye (fluorescein) and the grains appear much larger with the filter system used than under ordinary light microscopy. This makes discrimination between a few and many grains more difficult. Resolution obtained with this technique is inferior to that of conventionally stained sections. However, the size of the immunoglobulin-containing cells (IgA or IgG) and their morphology (small, eccentric nucleus and spherical cytoplasmic outline) is consistent with their being plasma cells. We have never found immunoglobulin present in macrophages. Further, in sections of the same mucosal biopsies stained with haematoxylin and eosin or Wright's stain, plasma cells abound and macrophages are rather rare.

Cells containing IgG antibody to intrinsic factor were found in two of the five biopsies tested with antihuman IgG (see Figs 1 and 2). In two of the biopsies, we could not be certain from the shape of the fluorescent cells underlying aggregations of grains that those cells were actually plasma cells. One of the biopsies, from a patient with a very high titre of circulating antibody to intrinsic factor, had so many grains in interstitial locations that it was not possible to be certain that any collection of grains was overlying a specific cell by the present technique. No autoantibody-containing cell of the IgA immunoglobulin type was found in any of the biopsies. These results, along with the immunoglobulin types of autoantibody to intrinsic factor in serum and gastric juice, are shown in Table 1.

Additionally, we noted a difference in the location of the IgG- and IgA-containing cells in the gastric mucosa of our five patients. The IgA-containing cells were found primarily in the lamina propria between the gastric glands while the IgG-containing cells were found predominantly in the lamina propria, deep to the glands, in the muscularis mucosae and in the submucosa when present.

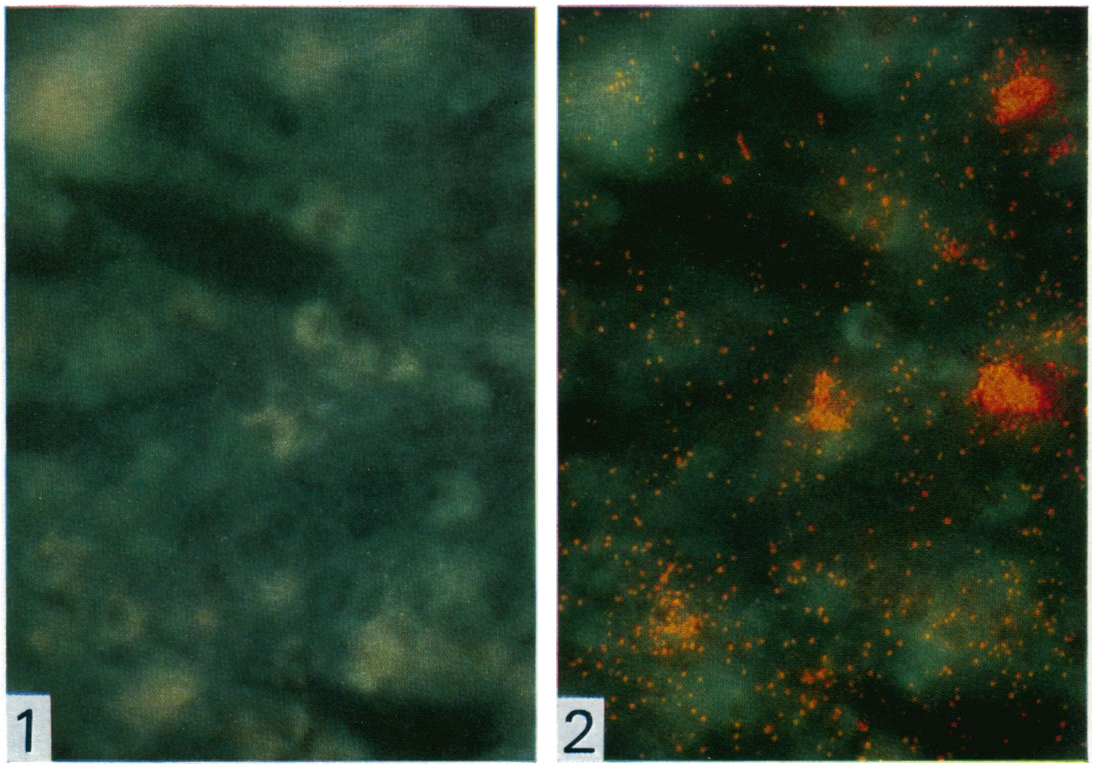

FIG. 1. Section of biopsy of inflamed gastric mucosa treated with fluoresceinated anti-human IgG and with a complex of $[^{57}Co]B_{12}$ and intrinsic factor. Photographed by U.V. light to show staining for IgG in plasma cells. × 600.

FIG. 2. A double exposure of the same section as in Fig. 1 examined first by U.V. fluorescence and subsequently for localization of $[^{57}Co]B_{12}$. A number of IgG-containing cells have autoantibody to intrinsic factor, and appear red. × 600.

(*Facing p.* 892)

TABLE 1

AUTOANTIBODIES TO IF-B_{12} COMPLEX IN PATIENTS WITH PERNICIOUS ANAEMIA

Patient	Inflammatory cells of mucosa		Serum		Gastric juice	
	IgG	IgA	IgG	IgA	IgG	IgA
1	+	−	+	+	+	−
2	+	−	+	+	+	+
3	±	−	+	−	+	+
4	±	−	+	−	+	−
5	−	−	+	+	+	+

DISCUSSION

IgA-containing mononuclear cells have been shown to predominate over IgG- and IgM-containing cells in the healthy gut (Gelzayd, Kraft and Kirsner, 1968; Crabbé and Heremans, 1966) and IgA is the predominant immunoglobulin in intestinal secretions in the healthy subject (Tomasi, Tan, Solomon and Predergast, 1965). Per oral immunization to an infective agent in man induces the formation of a specific IgA antibody in the gut secretions. Such antibodies are not formed following parenteral innoculation (Ogra, Karzon, Righthand and MacGillivray, 1968). Similarly, in germ-free mice, plasma cells containing a specific IgA antibody to a foreign protein can be induced in the gut and mesenteric lymph nodes in much larger numbers by oral than by parenteral immunization (Crabbé, Nash, Bazin, Eyssen and Heremans, 1969). In allergy to an orally ingested protein, gluten, in adult coeliac disease, inflammatory cells of the intestinal mucosa are primarily of the IgA type (Rubin, Frauci, Sleisenger, Jeffries and Margolis, 1965). Thus, there can be little doubt that IgA-inflammatory cells play a predominant role in formation of antibodies to ingested proteins. In contrast, in autoimmune diseases such as pernicious anaemia and ulcerative colitis the ratio of IgG- to IgA-containing cells is reversed in the mucosa of the stomach (Odgers and Wangel, 1968) and rectum (Gelzayd, Kraft, Fitch and Kirsner, 1968) respectively. We have now demonstrated IgG autoantibodies in inflammatory cells of the gastric mucosa, while no IgA autoantibody-containing cells were found. It is possible that by surveying more cells with the same technique we may find IgA-autoantibody-containing cells in the gut. Our data, however, suggest that the mechanism of production of autoantibodies in the gastrointestinal tract may not be the same as that of antibody formation to ingested agents.

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