Immunohistochemical observations of immunoglobulin A in the Paneth cells of germ-free and formerly-germ-free rats

Y. Satoh 1 *, K. Ishikawa 1, H. Tanaka 2, and K. Ono 1

1 Department of Anatomy, and 2 Animal Laboratory for Medical Research, Asahikawa Medical College, Nishikagura 4–5, Asahikawa, 078 Japan

Received January 17, 1986 / Accepted March 15, 1986

Summary. The localization of secretory immunoglobulin A (IgA) in Paneth cells was immunohistochemically studied in germ-free (Gf) and ex-Gf rats that had been injected with feces obtained from specific-pathogen-free (SPF) rats. In Gf as well as SPF rats, the secretory granules of Paneth cells and the brush borders of crypt cells exhibited IgA immunoreactivity. At 12 and 24 h after inoculation, it was found that, concomitant with the occurrence of considerable degranulation, the IgA immunoreactivity in Paneth cells disappeared, except of the margin of supranuclear vacuoles. In contrast, the IgA immunoreactivity of the crypt-cell brush borders was unchanged. Four days after inoculation, secretory granules exhibiting IgA immunoreactivity reaccumulated in Paneth cells. The present study suggests that Paneth cells regulate the bacterial milieu in the intestine by releasing secretory granules containing IgA into the crypt lumen.

Introduction

Paneth cells, which are pyramidal granulated cells found at the base of the crypts of the small intestine in many mammals (although not carnivora; for a review, see Otto 1974), were first observed by Schwalbe in 1872. Since then, various functions have been attributed to these cells, e.g., the secretion of digestive enzymes (Lewin 1969; Bohe et al. 1984; Senegas-Balas et al. 1984), the elimination of heavy metals (Halbhuber et al. 1970; Elmes 1976; Mottet and Bödy 1976; Phillpotts 1984), and the phagocytosis of intestinal microorganisms (Erlandsen and Chase 1972a, b).

It is becoming increasingly clear that Paneth cells play a role in controlling the bacterial milieu of the intestine by releasing lysozyme from their secretory granules (Deckx et al. 1967; Geyer 1973; Klockars and Osserman 1974; Otto 1974; Erlandsen et al. 1974; Peeters and Vantrappen 1975; Heitz and Wegmann 1980; Lopez-Lewellyn and Erlandsen 1980; Senegas-Balas et al. 1984). Lysozyme (muramidase, EC 3.2.1.17) may function as an antibacterial agent by destroying certain mucoproteins present in bacterial cell walls (McNabb and Tomasi 1981). Furthermore, the presence of immunoreactivity of immunoglobulin A (IgA), which is the predominant immunoglobulin in exocrine fluids, has been demonstrated in normal human and rat Paneth cells at the light-microscope level (Erlandsen et al. 1976; Rodning et al. 1976, 1982). However, in an electron-microscopic study, Brown et al. (1976) found no IgA immunoreactivity in human Paneth cells.

Recently, it has been reported that germ-free (Gf) rats subjected to the intraintestinal administration of bacteria-containing feces obtained from conventionally reared specific-pathogen-free (SPF) rats responded by exhibiting a massive decrease in the number of secretory granules present in Paneth cells. This experimental model is well suited for examining the histophysiology of Paneth cells (Satoh and Vollrath 1986; Satoh et al. 1986).

To gain more insight into the functions of Paneth cells, we compared IgA immunoreactivity in Paneth cells of Gf and ex-Gf (ExGf) rats with Paneth cell IgA immunoreactivity in SPF rats. For the localization of IgA in Paneth cells, plastic semithin sections gave better results than paraffin sections, this being due to their thinness, their more successful fixation (see Materials and methods), and the presence of fewer sectioning artifacts; however, in plastic sections, the antigenity was somewhat diminished. Therefore, in the present study, we used both semithin Epon and the paraffin sections.

Materials and methods

Twenty-five Gf (Jcl:Wistar; GN; Clea Japan) and 11 SPF (Jcl: Wistar) rats were used. All of the rats were males and were aged 10 weeks when killed. The Gf rats were randomly divided into five groups containing five rats. Three groups were inoculated with feces from SPF rats 4 days, 24 h, and 12 h before being killed (ExGf rats; cf. Satoh et al. 1986); the other two groups served as controls (Gf rats). To avoid diurnal variations, all of the animals were anesthetized with pentobarbital (25 mg/kg; given i.p.) between 09:00 and 12:00 a.m.

For paraffin-embedding procedures, five Gf and four SPF rats were perfused with Bouin's fixative (without acetic acid) via the left cardiac ventricle, and the terminal ileum was then removed. After immersion in Bouin's fixative for 12 h, the specimens were dehydrated in a graded series of ethanols, embedded in paraffin, and sectioned about 4 μm thick.

For Epon-embedding procedures, five Gf and four SPF rats were perfused with Bouin's fixative (without acetic acid) via the left cardiac ventricle, and the terminal ileum was then removed. After immersion in Bouin's fixative for 12 h, the specimens were dehydrated and embedded in Epon.
From each specimen, a few semithin sections (about 1 µm thick) were cut using a Reichert OmU3 ultramicrotome; one of these sections was stained with toluidine blue, while the others (used for immunohistochemical investigation) were immersed in 3% NaOH solution in absolute ethanol for 15 min in order to remove the Epon.

For immunohistochemistry, the peroxidase-antiperoxidase (PAP) procedure was performed according to the method of Sternberger (1986). Deparaffinized sections and semithin sections were etched for 10 min with 0.3% hydrogen peroxide in methanol in order to remove endogenous peroxidase activity, and the sections were then incubated for 30 min with 2% nonimmune rabbit serum in order to reduce nonspecific background staining. The sections were then incubated overnight at room temperature with goat antiserum to secretory IgA from rat bile (code no. 643511; Miles Scientific, Naperville) which had been appropriately diluted (1:200 for semithin sections; 1:2,000 for deparaffinized sections) with 0.01 mol/l phosphate buffer (pH 7.3) containing 0.5% Triton X-100. This was followed by incubations with unlabeled rabbit anti-goat IgG (code no. 65118; Miles Scientific; diluted 1:40) and the PAP complex (code no. B157, Dakopatts, Copenhagen; diluted 1:50) for 1 h each at room temperature. Subsequently, the sections were developed for 5-10 min in 0.0125% 3,3'-diaminobenzidine containing 0.002% hydrogen peroxide in 0.05 mol/l Tris-HCl buffer (pH 7.6). The specificity of the immunohistochemical staining was confirmed by replacing the primary antiserum with nonimmune rabbit serum.

Results

In paraffin sections, the Paneth cells located at the bottom of the intestinal crypts of SPF rats exhibited intense IgA immunoreactivity (Fig. 1). However the intensity of the staining for IgA varied in these Paneth cells, as previously reported by Erlandsen et al. (1976). Similar IgA immunoreactivity was observed in the Paneth cells of Gf rats (Fig. 2). Numerous plasma cells containing IgA were observed in the lamina propria in the ileum of SPF rats, but not of Gf rats. Prominent IgA immunoreactivity was observed in the Golgi area of crypt and villus epithelial cells in Gf rats, this being similar to that found in SPF rats.

In semithin sections stained with toluidine blue, the localization of Paneth cells, which were identified by the presence of secretory granules, was comparable to that observed in adjacent sections stained for IgA (Figs. 3-6). In SPF rats, Paneth cells contained numerous secretory granules (diameter, about 1–2 µm) that exhibited varying staining intensity for osmium and toluidine blue (Fig. 3a). In adjacent semithin sections incubated for immunohistochemical investigation, IgA was found in Paneth-cell granules and their margins; however, there was some variation in the number and staining intensity of the secretory granules located in individual and adjacent Paneth cells (Fig. 3b). The cytoplasmic matrix of some Paneth cells exhibited weak immunohistochemical staining for IgA. IgA immunoreactivity was observed in the luminal contents and brush borders of crypt cells, including Paneth cells; however, such immunoreactivity was not seen in the Golgi area of crypt cells, even though this site was stained for IgA in paraffin sections.

In Gf rats, the Paneth cells also contained many secretory granules (Fig. 4a), and the localization of IgA immunoreactivity was similar to that seen in SPF rats, i.e., some Paneth-cell granules as well as the brush border of crypt cells were stained for IgA, but the Golgi area of crypt cells was not (Fig. 4b). At 12 and 24 h after the inoculation of bacteria (ExGf rats), most Paneth cells exhibited considerable degranulation, and large supranuclear vacuoles (diameter, about 3–6 µm) were often observed in Paneth cells (Fig. 5a). No IgA immunoreactivity was observed in degranulated Paneth cells, except at the margin of the vacuoles. On the other hand, on the luminal surface of crypt cells, IgA was present (Fig. 5b). Four days after the inoculation of bacteria, the large secretory granules exhibiting IgA immunoreactivity had accumulated again (Fig. 6a, b).