Paneth Cell Identification in the Small Intestine of Guinea Pig Offsprings (*Cavia porcellus*)

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ABSTRACT

The aim of this study was to determine the presence, number, and morphometrical characteristics of Paneth cells (PC) in the small intestine of guinea pigs during lactation. We used 48 pups from 0 to 15 days old. Samples from small intestine were fixed in 10% buffered formaldehyde (pH 7.4) and processed for histological and morphometrical studies using hematoxylin and eosin (HE), Phloxine tartrazine or Masson's Trichome staining, or immunohistochemistry for lysozyme. PC were morphologically identified at day 2 using Masson's Trichome or Phloxine tartrazine stainings, and at day 4 using HE, whereas using immunohistochemistry they were recognized from birth. Morphometrical differences were found between the intestinal sections at each age studied, and within each section during the first weeks of life. In all developmental stage, the highest number of PC was observed in the duodenum of 13 days old guinea pigs. Our results confirm the presence of PC in the small intestine of guinea pigs from birth. Anat Rec, 297:856–863, 2014. © 2014 Wiley Periodicals, Inc.

Key words: Paneth cells; guinea pig; lactation; immunohistochemistry; lysozyme

INTRODUCTION

Guinea pig (*Cavia porcellus*) is a native rodent of the Peruvian Andes, Ecuador, Colombia, and Bolivia. It was originally domesticated for food production, due to the high nutritional characteristic of its meat and the low productive cost, as well as its hardiness, short life cycle and good fertility (Aguilar et al., 2011). Today, breeding and commercialization of guinea pigs are in growing demand in the world, and lactation is a critical stage

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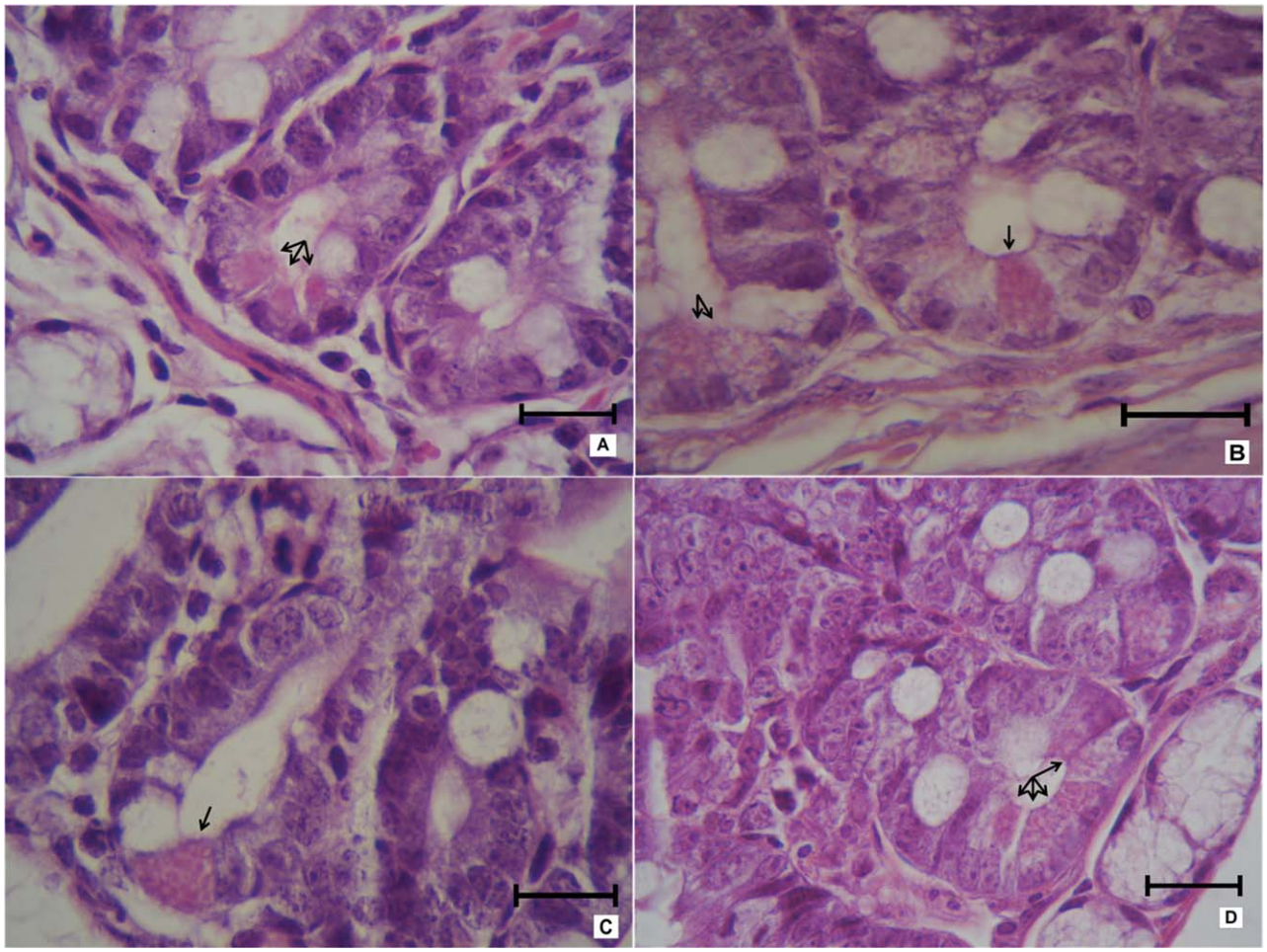

Fig. 1. Paneth cells (arrows) stained by HE. (**A**) Duodenum of a 6-days-old guinea pig, (**B**) jejunum of a 6 days old guinea pig, (**C**) ileum of a 6-days-old guinea pig, (**D**) duodenum of a 15-days-old guinea pig. Scale bars for A–D = 20 μm.

for that purpose. During lactation guinea pigs are more susceptible to diverse infectious agents that may delay their growth or cause their death, especially during the first 2 weeks of life. In Peru, the neonatal mortality in commercially raised animals is about 59%, and 72% of these deaths are due to enteric problems, mainly those caused by the bacterium *Salmonella* (Cordero et al., 2001; Layme et al., 2011). However, little is known about general physiologycal aspects of the gastrointestinal tract, the intestinal innate immune response mechanisms, as well as the role of Paneth cells (PC) in young guinea pigs during lactation.

Paneth Cells are secretory epithelial cells located in the Lieberkühn crypts of the small intestine of most mammals (Porter et al., 2002). These cells originate from crypt base columnar intestinal stem cells and differentiate during a downward migration to reach their definite location at the bottom of the crypts (Garabedian et al., 1997). PC are pyramidal in shape with apical granules (Ouellette et al., 2000) that are rich in essential molecules associated with the innate immunity, such as α-defensins (Cunliffe and Mahida, 2004), lysozyme (Coutinho et al., 1998; Hecht, 1999; Hornef et al., 2004), phospholipase A2 (Harwig et al., 1995), and other substances involved in the regulation of intestinal endogenous flora composition, cell proliferation, and digestive and detoxification processes (Bevins, 2004; Ouellette, 2005; Porter et al., 2002). PC contribute to host defense against enteric pathogens and thus they participate in the maintenance of the intestinal homeostasis. It has been shown that mice deficient in specific α-defensins are more susceptible to *Salmonella enterica serovar typhimurium* infection (Wilson et al., 1999), whereas the expression of human α-defensins in transgenic mice reduces this susceptibility (Salzman et al., 2003). Recent studies support the existence of a functional interaction between PC and crypt stem cells and, therefore, they are considered members of the stem cell niche. PC provides essential signals to crypt base columnar stem cells and modulate their biology in the healthy intestine. Besides, PC are sensors of the nutritional status of the organism, and they can respond to caloric restriction through the secretion of substances that modulate the stem cell biology (Yilmaz et al., 2012; Clevers and Bevins, 2013). In addition, PC plays a relevant role in the pathogenesis of intestinal diseases, such as those induced by infectious agents, and they participate in repairing the intestine after damage (McElroy et al., 2013).

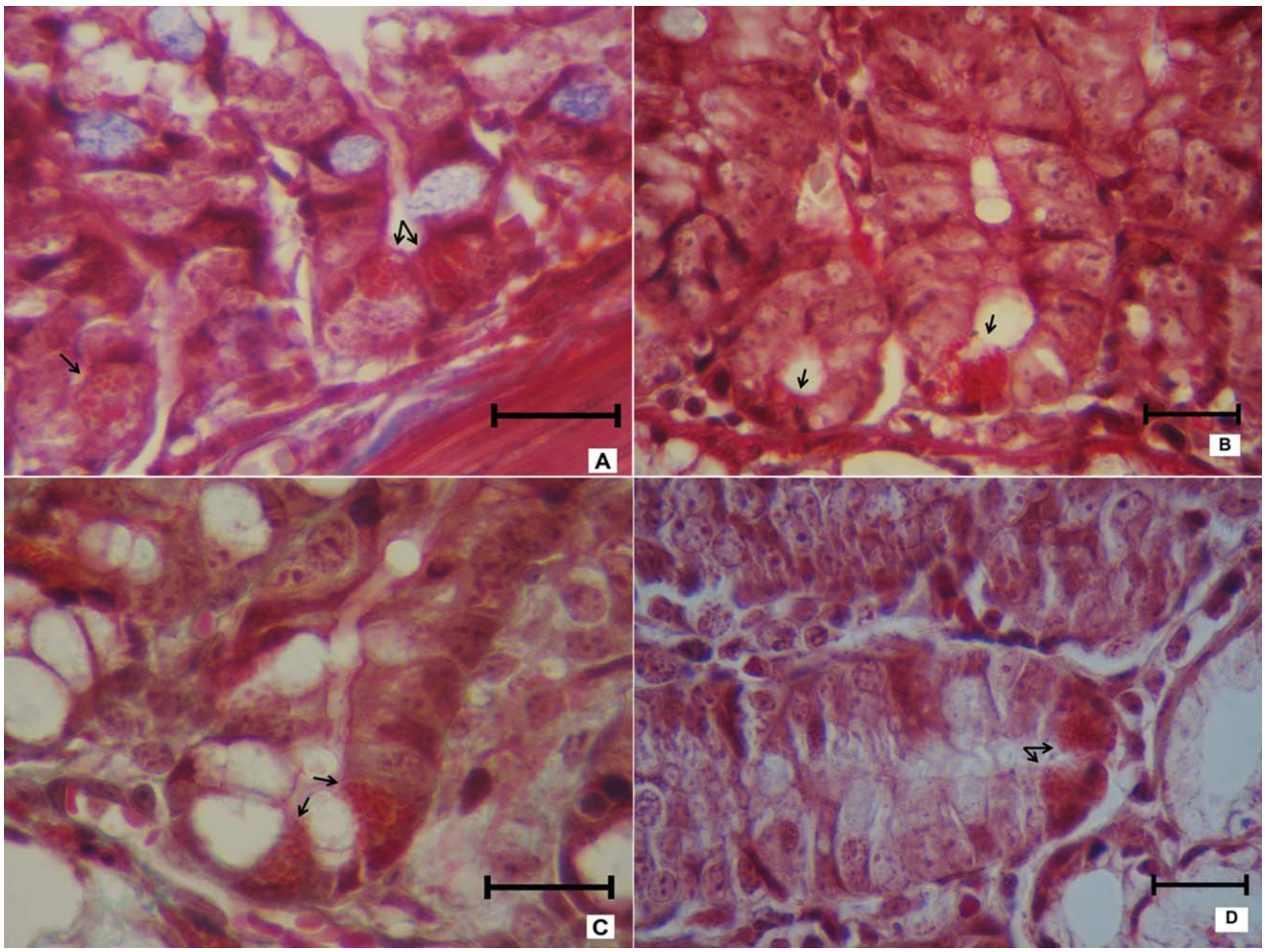

Fig. 2. Paneth cells stained by Masson's Trichome. Note the red staining of PC granules (arrows). (**A**) Jejunum of a neonate, (**B**) duodenum of a 6-days-old guinea pig, (**C**) ileum a 6-days-old guinea pig, (**D**) duodenum of a 13-days-old guinea pig. Scale bars for A–D = 20 μm.

Histochemical and morphological studies have shown the presence of PC in several species, such as rat, mouse (Rodning et al., 1982; Sinke and Geyev, 1968), golden hamster, human being (Ehrmann et al., 1990; Lewin, 1969a, b), rabbit (Oestrich et al., 1970; Zanuzzi et al., 2008), squirrel (Toth, 1980), horse (Takehana et al., 1998), sheep (Ergün et al., 2003), and alpaca (Lira et al., 2012). In guinea pigs PC were also identified, but only in adults (Satoh et al., 1990; Tsumura et al., 1998).

Aim of Work

Taking into account the relevant role of PC in the gastrointestinal immunity of other species, and their involvement in some inflammatory intestinal diseases, we aimed to identify PC in guinea pigs during lactation, and describe their morphometrical and histochemical characteristics.

MATERIALS AND METHODS

2.1.Animals

Forty-eight Line 1 La Molina guinea pigs (from 0 to 15 days old) were used in this study**.** All animals were clinically healthy at birth. All animals were kept in a 12:12 light dark cycle with free access to food and water. All the management and experimental procedures were carried out according to national regulations, followed to the 'Guide for the Care and Use of Laboratory Animals' (National Academy Press, 1996, Washington, DC).

Histological and Histochemical Studies

Samples of 2 cm long of duodenum, jejunum, and ileum were collected from each animal, rinsed in PBS, fixed in 10% buffered formaldehyde (pH 7.4) and embedded in paraffin. Slices of 5 μm thick were stained hematoxylin and eosin (HE) (Stevens, 1990), Phloxine tartrazine (AFIP, 1995) or Masson's Trichome stainings (Bradbury and Gordon, 1990) or used for immunohistochemistry (Zanuzzi et al., 2008).

Immunohistochemistry

Five μm sections were mounted on slides coated with poli-L-lysine- (P 8920, Sigma-Aldrich), and passed through a decreasing graded alcohol scale, and incubated with 3%

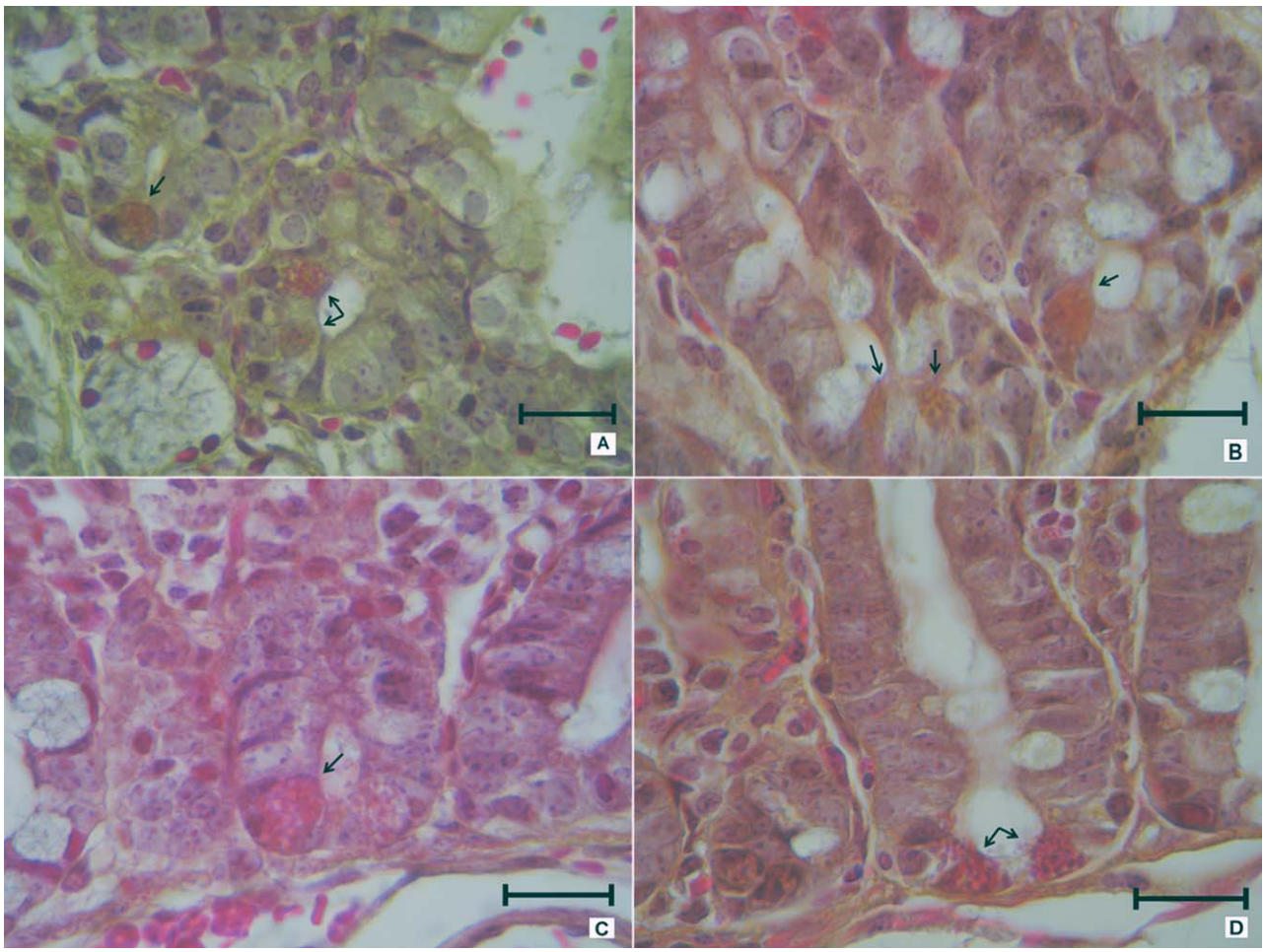

Fig. 3. Paneth cells (arrows) stained by Phloxine tartrazine. (**A**) Duodenum of a neonate, (**B**) jejunum of a 6-days-old guinea pig, (**C**) ileum of a 6-days-old guinea pig, and (**D**) duodenum of a 15-days-old guinea pig (B). Scale bars for A–D = 20 μm.

H_2O_2 in methanol for 30 min at room temperature to inhibit endogenous peroxidase activity. Antigen (Ag) retrieval was performed in citrate buffer (pH 6.0). Nonspecific binding sites were blocked with 1% bovine serum albumin (BSA) for 30 min followed by the overnight incubation with the primary biotinylated rabbit polyclonal antihuman lysozyme antibody (Accurate Chemical and Scientific Corporation, Westbury, NY) diluted at 1:50. Horseradish peroxidase streptavidin SA 704 (Vector Laboratories, Burlingame, CA) was used as a detection system and incubated for 30 min. Slides were rinsed twice in PBS for 5 min, then revealed with liquid 3,3′-diaminobenzidine tetrahydrochloride as chromogen (Dako Cytomation) and counterstained with Harris' hematoxylin (Lira et al., 2012; Zanuzzi et al., 2008). The dark, golden brown DAB hydrogen peroxide reaction product showed the positively stained structures. Positive control samples for the immunohistochemical detection of lysozyme included: lachrymal gland of mouse and guinea pig, and jejunum and ileum of an adult mouse. As negative control, slides were incubated with normal rabbit serum instead of the primary antibody.

Image Analysis

The histological description and quantification of morphometric parameters were carried out by digital image analysis with the program AxioVision LE Canon. Images were captured (10, 20, 40, or 100× amplification) and digitized in TIFF format. Images from HE stained 5-μm sections were captured with an objective magnification of 40×. To evaluate cell area and major and minor axis fifteen randomly PC of each intestinal section were measured. Paneth cell number per section of crypt was also evaluated. For this purpose, 50 sections of crypts per animal were evaluated, considering only those fully visible and perpendicular to the muscularis mucosae. Digital images were then analyzed using the Manual Tag function of the image analysis software. Raw data were exported to a spreadsheet in order to perform statistical analysis.

Statistical Analysis

Statistical analysis was performed using SPS 15.0 program. The ANOVA test was used to evaluate differences among groups, followed by multiple comparisons Tukey's

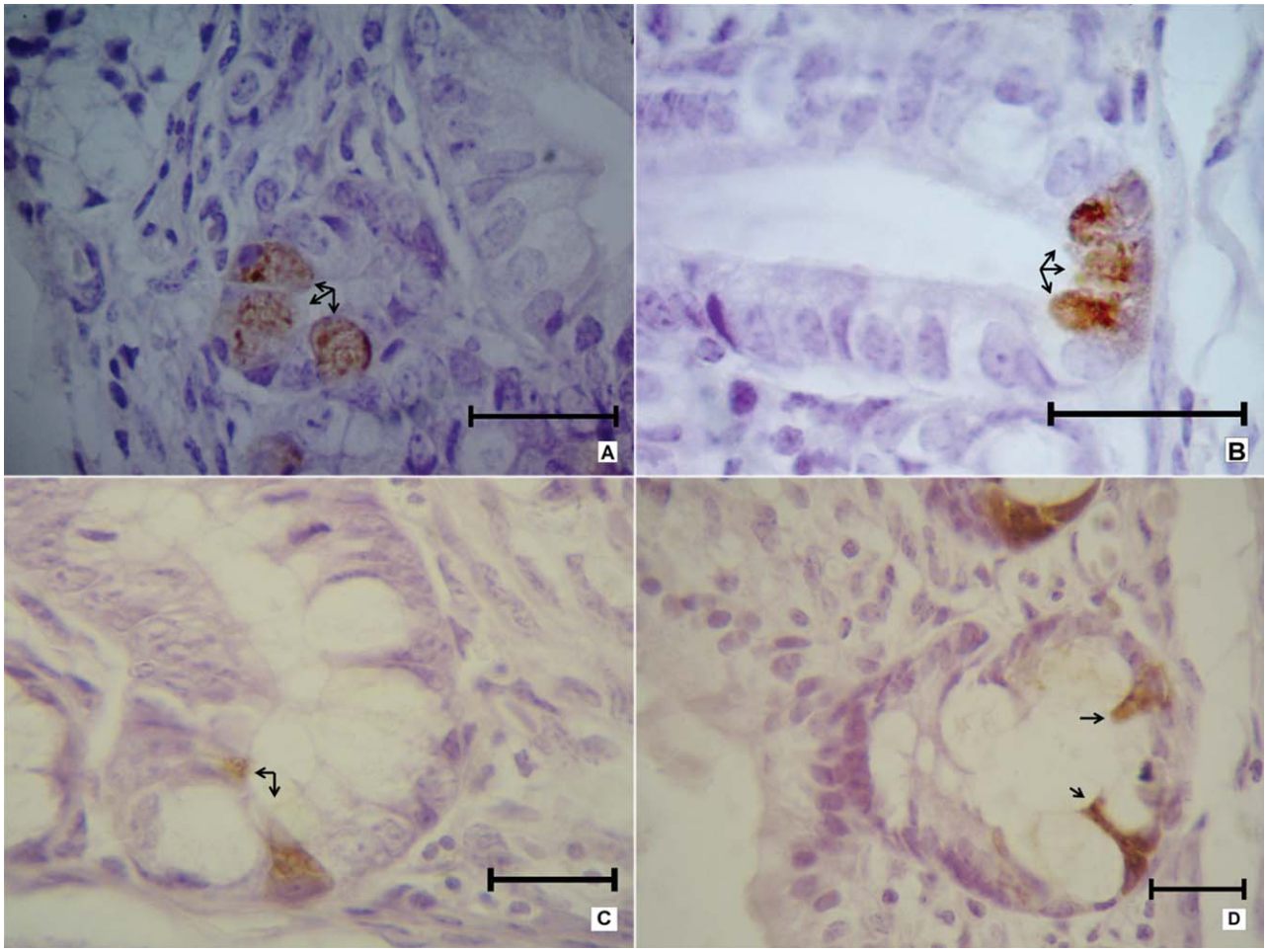

Fig. 4. Immunohistochemical detection of lysozyme in Paneth cells' granules (arrows). (**A**) Duodenum of a neonate, (**B**) jejunum of a 6-days-old guinea pig, (**C**) ileum a 13-days-old guinea pig, (**D**) ileum a 15-days-old guinea pig. Scale bars for A–D = 20 μm.

test. Significant differences were defined as those with $P < 0.05$.

RESULTS

Histological and Histochemical Characteristics

In HE stained sections PC were recognized at the bottom of the Lieberkühn glands in the three intestinal segments of 4-days-old animals (Fig. 1), whereas using Masson's trichrome (Fig. 2) or phloxine tartrazine stainings (Fig. 3) they were identified at day 0 (neonate). Inmmunohistochemical detection of lysozyme was positive from birth in all the intestinal segments.

PC were pyramidal in shape with apical granules in the samples studied (Fig. 1). The granules were eosinophilic, stained red with Phloxine tartrazine or Masson's Trichromic staining (Figs. 2 and 3), and immunohistochemically positive to lysozyme in all the intestinal sections and studied ages (Fig. 4).

Morphometry

Mean major axis of PC did not differ between intestinal sections of each animal or between animals from birth up to day 15 of age. However, mean minor axis value was significantly higher in the duodenum than in the ileum and jejunum (8.84 ± 1.7 vs. 8.39 ± 1.7 μm and 8.08 ± 1.2 μm, respectively). Besides, PC area was higher in the duodenum and ileum at birth, and then it decreased to finally increase up to day 15 of age (Fig. 5A–C).

PC number increased from birth up to day 11 in the jejunum and ileum, whereas in the duodenum it increased was up to day 13. Statistical significant differences were found between the first and posnatal day 15 between intestinal sections ($P < 0.05$) (Fig. 5A).

DISCUSSION

Previous studies have shown the presence of PC in adult guinea pigs (Satoh et al., 1990; Tsumura et al., 1998). In the present work, we used different techniques and recognized PC at the bottom of the Lieberkühn glands of the duodenum, jejunum and ileum of young guinea pigs. Although the composition of the granules of PC differs between species, immunohistochemistry to detect lysozyme was the most specific and suitable method to recognize these cells from birth, as it has also

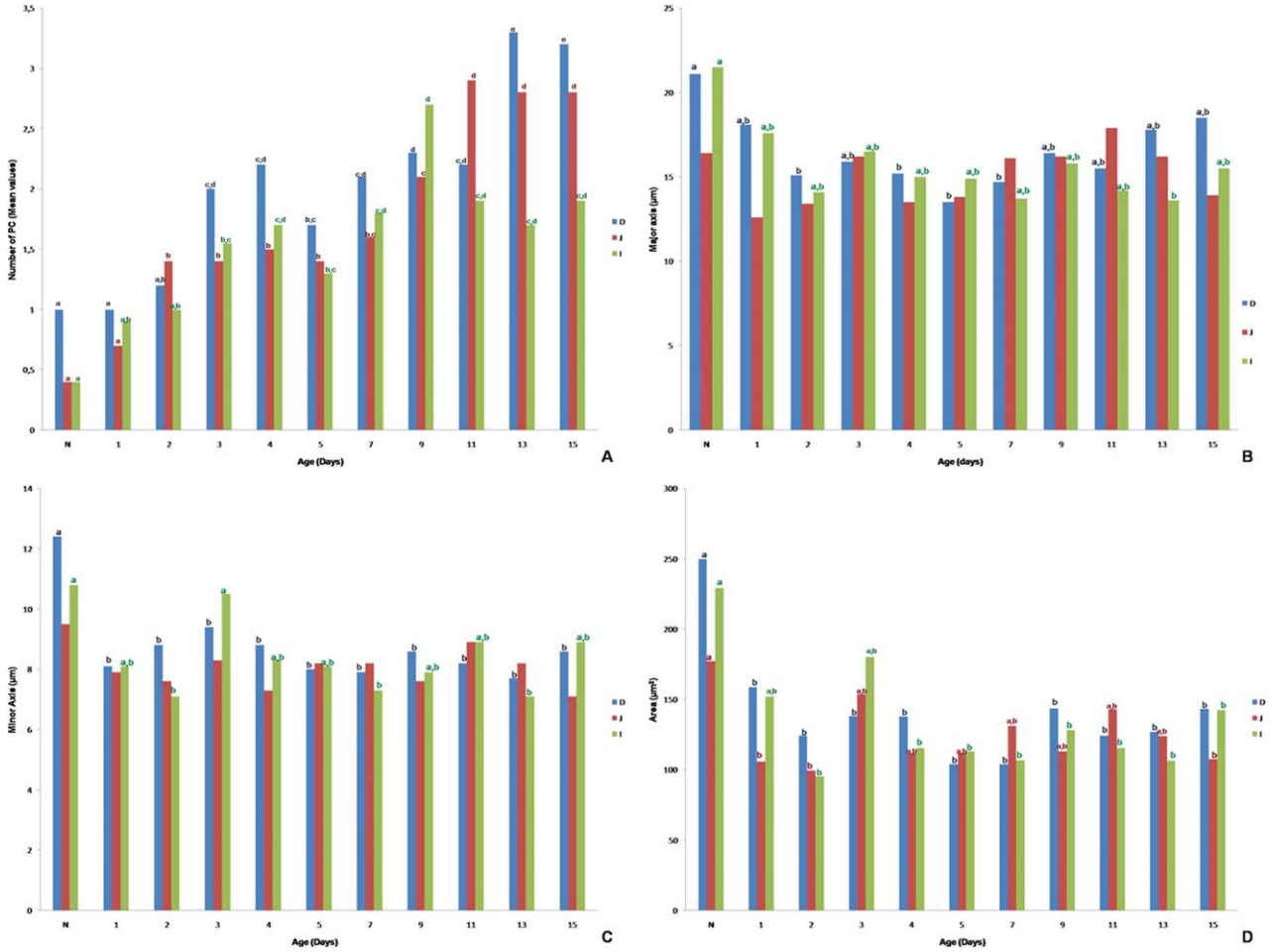

Fig. 5. (**A**) Paneth cell number in the small intestine of guinea pigs. (**A–E**) Different letters show significant statistical differences ($P < 0.05$) between different ages of each intestinal section. Paneth cell morphometry in the small intestine of guinea pigs. (**B**) Major axis, (**C**) minor axis, (**D**) area. D: duodenum; J: jejunum; I: ileum.

been shown by other studies (Peeters and Vantrapper, 1975; Garabedian et al., 1997; Satot et al., 2009).

Up to now, little is known about PC in adult or young guinea pigs, and we have not found references regarding PC in suckling animals. We found some differences with other species better studied, such as mice. In mice, intestinal crypts formation occurs during the first 2 weeks of postnatal life (Cheng and Bjerknes, 1985). The morphological identification of PC in this species is evident at day 7 of posnatal development (Bry et al., 1994). However, positive cells for cryptydin were found earlier, at day 15 of prenatal life. In the intestine of guinea pigs the crypts are well-developed at birth (Bayley et al., 1984) and the recognition of PC in pups suggests that they may begin their differentiation during the prenatal stage (Ouellette et al., 2000; Putsep et al., 2000). It is likely that precocial mode of development of guinea pigs explains the earlier morphological identification of PC in this species, in contrast to the altricial mode of mice. In addition, in mice and guinea pigs PC are restricted to the base of the crypts. This location seems to be necessary to finally obtain their definitive morphological and functional characteristics, since their differentiation depends on the reciprocal induction between epithelial crypt cells and other cells, such as pericryptal myofibroblasts (Keshav, 2006).

We described an increase in PC number in all the intestinal sections from birth up to day 15. This increase is in accordance with the results reported in mice by Bry et al. (1994). We have not found morphometrical studies of PC in guinea pigs during lactation, and there are many controversial data of PC in adults from different species. Thus, in sheep Ergün et al. (2003) reported a mean number of 2.49 PC per crypt in the jejunum, whereas in rabbits Zanuzzi et al. (2008) found 4.28 PC per crypt. In contrast, Cheng and Leblond (1974) reported between 5 and 12 PC in the small intestine of mice. We suggest that differences in the methodological criteria selected or adapted for each work may have influenced those results. In addition, specie-specific variations may also be taken into consideration. Although we did not quantify the number of PC in weaned and adult guinea pigs, we hypothesize that an increase in its number during the first weeks of posnatal life may be in accordance with the maturity state of the animal.

The mean morphometrical values for PC in pups of guinea pigs were higher than those reported in alpacas

by Lira et al. (2012), and in rabbits by Zanuzzi et al. (2008). These differences may be associated not only with the species but also with the age of the animals. The increase in PC number in pups of guinea pigs is associated with a lower size of the cells. This increase may be related to animal growth and development, a fact that may explain the differences found between species (Cheng and Leblond, 1974; Ergün et al. 2003; Zanuzzi et al., 2008). We suggest that the increase in the number of PC in rodents may begin at first stages of life, independently of the level of intestinal development at birth. In addition, the significantly decreased of the area of PC observed in pups of postnatal days 1 and 2 may be due to the exposition to new intestinal luminal content, in which nutrients and orally acquired microbes are present. Thus, under this new environment PC become active and exocytose the stored products of their granules, and thus they reduce in size during the first pos natal days. However, even after 15 days from birth, PC area did not reach the area values found at birth. A likely explanation is that PC has adapted to the luminal content of the intestine and reached a balanced between secretory and exocytic activity. These results may indicate the functionality of PC at this early age in guinea pigs, and support the role as sensors of the nutritional status of the organism (Clevers and Bevins, 2013).

The presence of PC in suckling guinea pigs may create an antimicrobial protective intestinal barrier, rich in lysozyme and other substances (Elphick and Mahida, 2005) that may reduce their susceptibility to infections. It was shown that the number of PC and their secretion is increased in mice infected by *Salmonella enterica serovar typhimurium* (Martínez Rodríguez et al., 2012). The multifacetic functionality of PC also include their role as sensors of microbial invasion via surface and intracellular receptors that lead to the initiation of local and a systemic inflammatory response with the secretion of several cytokines, growth factors and vasoactive mediators. As this regard, it has been proposed a crucial role for PC in the pathogenesis of gastrointestinal disease of preterm infants, such as necrotizing enterocolitis (Mc Elroy et al., 2013). However, up to now, necrotizing enterocolitis induced by enterobacteria has not been reported in guinea pigs.

The presence of PC in young guinea pigs, in addition to the morphometrical changes described during the first 15 days of life, and the detection of lysozyme in their granules as reported here, support their early functionality. Therefore, since in guinea pigs PC are mature from birth they may be involved in the high neonatal mortality of this specie. Future studies will investigate the role of PC at weaning and after this stressful period in which animals are more susceptible to infectious diseases.

CONCLUSION

We show for the first time the presence of PC in the healthy intestine in young guinea pigs, and describe their morphometric characteristics. The present work will contribute to further understand possible morphological changes of PC under diverse pathological conditions and their role in the pathogenesis of those diseases.

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