In vivo killing of *Giardia* trophozoites harbouring bacterial endosymbionts by intestinal Paneth cells: an ultrastructural study

K. A. EL-SHEWY[1]* *and* R. A. EID[2]

[1] *Microbiology and Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[2] *Electron Microscopy Center, Faculty of Sciences, Zagazig University, Egypt*

(*Received 1 January 2004; revised 10 August 2004; accepted 10 August 2004*)

SUMMARY

To date Paneth cells have not previously been reported to kill *Giardia* trophozoites and other protozoa *in vivo*. Here we report the first evidence for *in vivo* killing of *Giardia* trophozoites by intestinal Paneth cells. Transmission electron microscopic (TEM) examination of duodenal specimens taken from naturally infected mice revealed that only *Giardia* trophozoites harbouring peripheral bacterial endosymbionts were destroyed and lysed in the vicinity of the activated Paneth cells. Additionally, intestinal epithelium was more affected by *Giardia* harbouring bacterial endosymbionts than *Giardia* with no endosymbionts. Our findings imply that the bacterial endosymbionts within *Giardia* trophozoites have a role in both host protective and pathological mechanisms, probably through altering the trophozoite antigencity. These observations might shed light on the diversity in infectivity and host specificity of *Giardia* species.

Key words: endosymbiosis, giardiasis, electron microscopy, Paneth cells, *in vivo* killing, naturally infected mice.

INTRODUCTION

Paneth cells are epithelial granulocytes located at the base of the crypts of Lieberkühn in the small intestine of many mammalian species (Ouellette & Selsted, 1996). These cells contribute actively to mucosal immunity through secretion of antimicrobial polypeptides including α-defensins (cryptdin) that are released from the intestinal Paneth cells in response to bacteria and bacterial antigens, but not to protozoa. These antimicrobial polypeptides kill a wide range of organisms including bacteria, viruses, fungi and tumour cells (Martin, Ganz & Lehrer, 1995; Ayabe *et al*. 2000).

Some *in vitro* studies have reported that *Giardia* trophozoites were destroyed and lysed by defensins and other antimicrobial polypeptides derived from the intestinal Paneth cells (Aley *et al*. 1994). However, no evidence has been reported so far for the *in vivo* killing of *Giardia* trophozoites when these cells are stimulated.

Giardia trophozoites, in common with some other protozoan parasites, demonstrate bacterial endosymbiosis (de-Souza & Motta, 1999). The significance of the bacterial endosymbionts within *Giardia* is not well defined. It is possible that they could alter trophozoite pathogencity, metabolism, range of infectivity and host specificity, as they do in other protozoa (Nemanic *et al*. 1979).

In this report the ultrastructural interaction between *Giardia* trophozoites harbouring bacterial endosymbionts and mouse intestinal Paneth cells is described.

MATERIALS AND METHODS

Animals

Light and transmission electron microscopic (TEM) examinations were conducted for examination of the duodenal specimens taken from *Giardia* infected mice. Twenty-five laboratory reared albino mice (Tuck ordinary strain), naturally infected with *Giardia muris* as detected by screening of their faecal suspension (Roberts-Thomson, Stevens & Mahmmoud, 1976), were chosen. It was found that 10 of these 25 mice were heavily infected with *G. muris* trophozoites. These heavily infected mice were sacrificed.

Mice were dissected and duodenal segments were removed. Segments were fixed in alcoholic Bouin with 10% buffered neutral formalin for subsequent light microscopic examination. Similar segments were fixed in 2·5% glutaraldehyde for TEM examination.

Transmission electron microscopy

Specimens prepared for TEM were cut into 2–3 mm^2 sections and immediately fixed in 2·5%

* Corresponding author: Microbiology Department, College of Medicine and Medical Sciences, King Khalid University, P.O. Box 641, Abha, Kingdom of Saudi Arabia. Fax: 00966 72247570. E-mail: Khel666@hotmail.com

DOI: 10.1017/S0031182004006547 Printed in the United Kingdom

glutaraldehyde with 0·1 M sodium cacodylate buffer (pH 7·2) at 4 °C for 2–3 h. Specimens were post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethyl alcohol, and embedded in Spurr's resin. Sections (0·5 μm) were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 1200 EX TEM at 80 KV (Glauert, 1980).

RESULTS

TEM examination of the specimens taken from naturally infected mice demonstrated many *Giardia* trophozoites on the brush border, at the base of the crypts as well as among the intestinal microvilli. The internal fine structure of these trophozoites was clearly demonstrated (Fig. 1A, B). Severe blunting and atrophy of microvilli with occasional loss of basic epithelial morphology and distorted Goblet cells were observed in some of the examined duodenal sections (Fig. 1C, D).

In these duodenal specimens bacterial endosymbionts within *Giardia* trophozoites were demonstrated. Bacteria were found in close contact with the outer surface of some *Giardia* trophozoites. In some others, they were inundating the trophozoite cell membrane. Electron-lucent spaces were detected surrounding the bacterial endosymbionts and bounded with aggregation of cytoplasmic granules (Fig. 2A, B). No evidence was found for digestion of these bacterial endosymbionts by *Giardia* trophozoites. There were, however, many electron-lucent vacuoles forming a track directed towards the periphery of the trophozoite. Endosymbionts were concentrated peripherally in direct contact with the intestinal microvilli, and were uncommon in the nuclear area (Fig. 2C).

No gross morphological changes were detected in the *Giardia* trophozoites as the result of these bacterial endosymbionts. However, *Giardia* trophozoites were found completely lysed at the base of the duodenal crypts and close to the Paneth cells (Fig. 3A–D).

Paneth cells were found to be highly active with many secretory granules inside, especially those in contact with *Giardia* trophozoites harbouring peripheral endosymbionts. On the other hand Paneth and Goblet cells associated with *Giardia* trophozoites and having no endosymbionts were found inactive and the trophozoites were demonstrated to be completely intact and discrete. Mucosal lining in close contact with such trophozoites was observed to be almost intact compared to the severely damaged mucosa observed with trophozoites containing endosymbionts (Figs 1D and 3E).

DISCUSSION

The precise nature of the interaction between *Giardia* and its host remains conjectural because of the paucity of published studies. The present ultrastructural report describes an interaction between *Giardia* trophozoites and intestinal Paneth cells in the presence of bacterial endosymbionts.

Although intestinal Paneth cells secrete antimicrobial peptides that affect many intestinal pathogens, *Giardia* as many other protozoan parasites have not previously been reported to be killed by Paneth cells or by their antimicrobial secretions (Jones & Bevins, 1992; Selsted *et al*. 1992; Aley *et al*. 1994).

In the present report we observed lysis of the cell membrane of *Giardia* trophozoites when bacterial endosymbionts came in close contact with Paneth cells. Intestinal Paneth cells associated with such trophozoites were found highly activated and with many internal secretory granules.

We could not detect lysis of any *Giardia* trophozoites free from bacterial endosymbionts and we therefore speculate that the bacterial endosymbionts stimulate Paneth cells. *Giardia* trophozoites did not stimulate Paneth cells when these cells were exposed to trophozoites *in vitro* (Ayabe *et al*. 2000). However, the authors did not exclude the possibility that these trophozoites and other eukaryotes may induce Paneth cell secretion by an unidentified mechanism *in vivo*.

The reported activation and lytic effect of Paneth cells were seen in all duodenal specimens examined with trophozoites harbouring bacterial endosymbionts. This lead to our proposal that bacterial endosymbionts activate Paneth cells, resulting in release of their lytic peptides mainly defensins. It is likely that defensins are responsible for *Giardia* lysis since they account for 70% of the released bactericidal activity of Paneth cells (Ayabe *et al*. 2000). In an earlier study, α-defensins demonstrated a remarkable lysis and killing of *Giardia* trophozoites *in vitro* (Aley *et al*. 1994).

The pathological changes observed in this study as the result of *Giardia* trophozoites indicated that intestinal epithelium was more affected by *Giardia* with bacterial endosymbionts than those without endosymbionts suggesting that bacterial endosymbionts might alter the trophozoite pathogenicity.

The reported variation in the pathogenicity and the stimulation of the Paneth cells to react actively with the trophozoites could be due to variation in the trophozoite surface antigens and consequently the immunopathological process (Meng, Hetsko & Gillin, 1993). Such variation in the *Giardia* surface antigens as the result of bacterial endosymbionts has been proposed earlier (Meyer, 1990).

Bacterial flora and other organisms that live in the intestine appear to provide the several necessary services for healthy development and the suggested functions of the bacterial endosymbionts described here could explain the significance of endosymbiosis in *Giardia* and other similar protozoa.

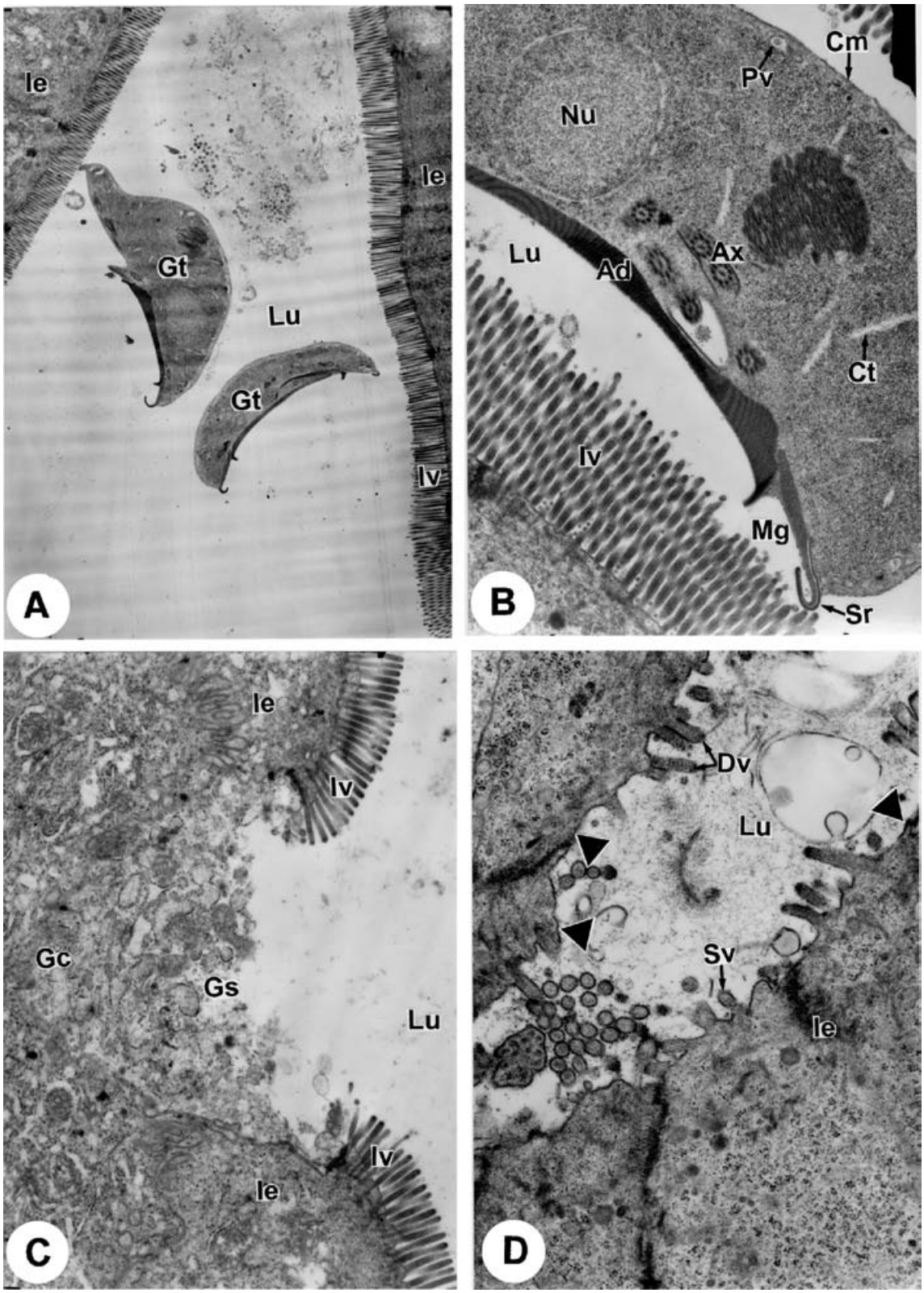

Fig. 1. (A) Transmission electron micrograph of duodenal specimen taken from naturally infected mice demonstrating intestinal microvilli (Iv) covering the intestinal epithelium (Ie), with *Giardia* trophozoites (Gt) in the intestinal lumen (Lu). (× 5000.) (B) Higher magnification of the *Giardia* trophozoite seen in (A) Adhesive disc (Ad), nucleus (N), peripheral vesicles (Pv), cell membrane (Cm), marginal groove (Mg), and the striated rim of the groove (Sr). Ax represents the axonemes of the flagellum, cytoplasmic tubules (Ct), intestinal microvilli (Iv), and lumen (Lu). (× 17 500.) (C) TEM of duodenal mucosa infected with *Giardia* trophozoites, demonstrating marked stimulation of the Goblet cell (Gc), with Goblet secretion (Gs), in between the intestinal epithelium (Ie) covered with microvilli (Iv). (× 9500.) (D) Severely damaged epithelial mucosa as the result of *Giardia* infection including shortened microvilli (Sv), distorted microvilli (Dv), and marked mucosal atrophy in between the affected microvilli (arrow heads). Intestinal epithelium (Ie). (× 20 700.)

https://doi.org/10.1017/S0031182004006547 Published online by Cambridge University Press

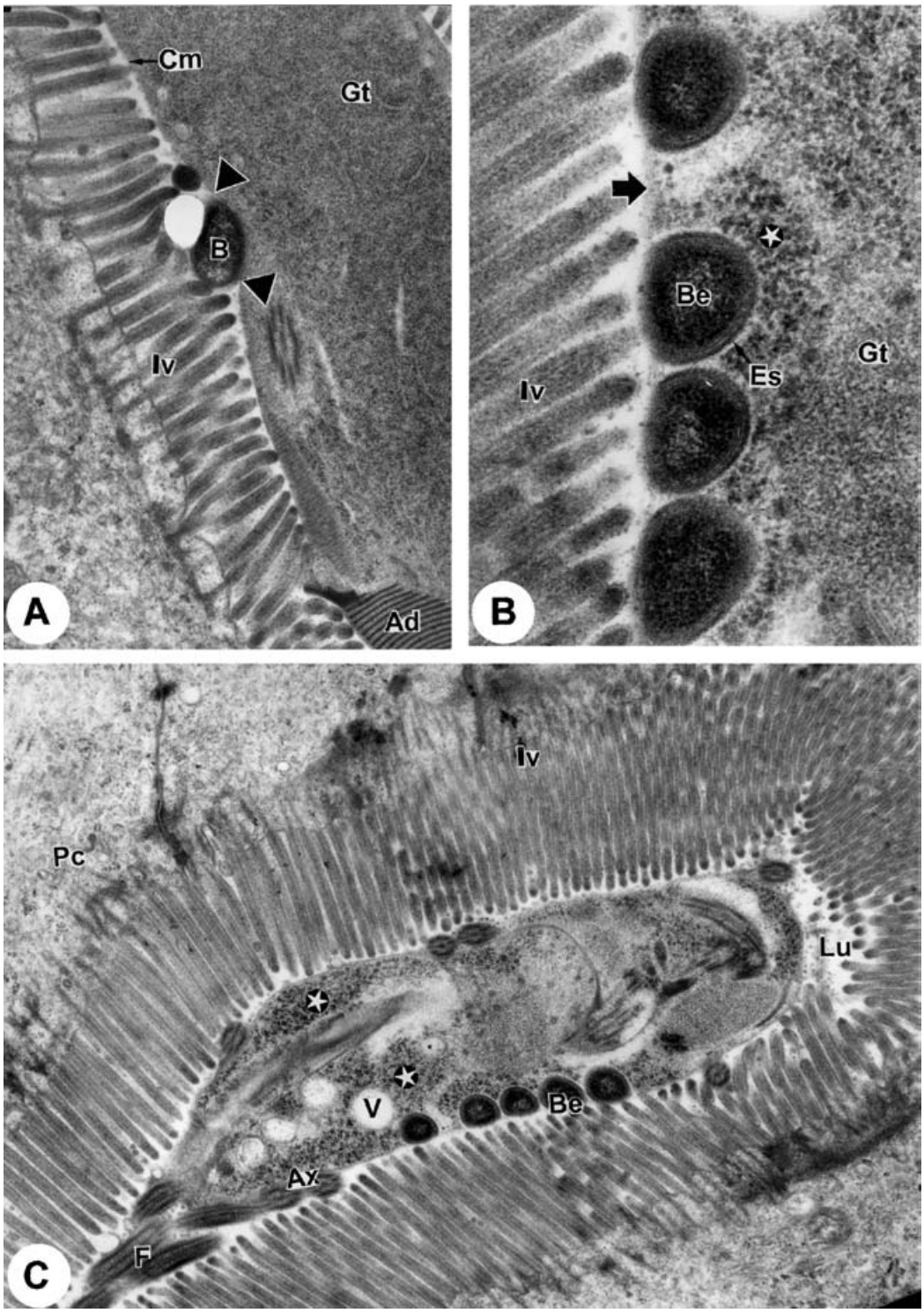

Fig. 2. (A) TEM picture of duodenal mucosa showing the first step in the bacterial endosymbiosis. B represents bacterial body in between the intestinal microvilli (Iv) and *Giardia* trophozoite (Gt). The bacterial body is pushing itself toward the cell membrane (Cm) of the trophozoite by making invagination in it (arrow heads). Adhesive disc (Ad). (× 27 500.) (B) Higher magnification of the *Giardia* trophozoite (Gt), with endosymbiosis, showing multiple bacterial endosymbionts (Be) completely invaginating inside the cell membrane of the *Giardia* trophozoite (arrows), and bounded with electron-lucent space (Es), and surrounded with many cytoplasmic granules (star). Note the peripherally location of endosymbionts. Iv, intestinal microvilli. (× 71 400.) (C) Longitudinal section of *Giardia* trophozoite showing multiple bacterial endosymbionts (Be), with a track of electro-lucent vacuoles directed towards the periphery of the trophozoites (V). Cytoplasmic granules (stars); Ax, represents axonemes of the flagellum (F); intestinal villi (Iv); Paneth cell (Pc). (× 20 000.)

https://doi.org/10.1017/S0031182004006547 Published online by Cambridge University Press

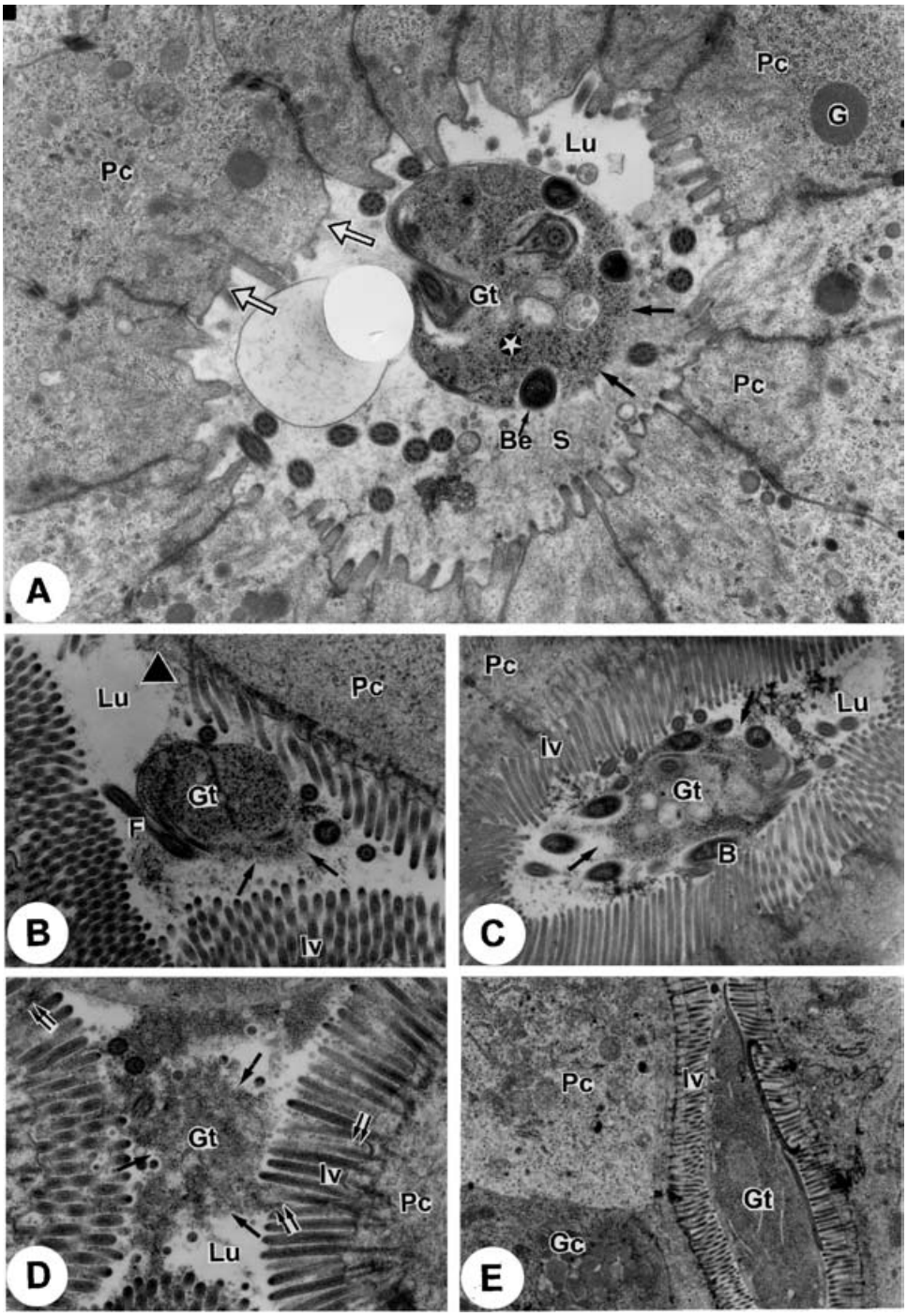

Fig. 3. (A) TEM picture of a transverse section in an intestinal crypt showing ultrastructural interactions between *Giardia* trophozoite, bacterial endosymbionts, and Paneth cells. Cell membrane of *Giardia* trophozoite (Gt) appears lysed at the dorsal surface of the parasite (black arrows) with peripheral bacterial endosymbionts (Be) in close contact with Paneth cells (Pc). The interface between the trophozoite and the cells is filled with a dense secretion (S). Paneth cells appear active with many secretory granules inside (G), granular cytoplasm (stars), damaged mucosa and severely distorted epithelial microvilli (white arrows); Lu, lumen. (× 17 500.) (B–D) Transverse sections of *Giardia* trophozoite (Gt) at the base of the intestinal crypt demonstrating different stages of lysis and destruction of the *Giardia* body by the surrounding Paneth cells (Pc). B, bacteria; lysed cell membrane (arrows); flagellum (F); atrophied mucosal lining (arrow head), with lysed trophozoites in between the microvilli (double arrows). (× 14 200 (B), 13 600 (C) and 20 000 (D).) (E) TEM picture demonstrating *Giardia* trophozoites (Gt) lodged in between the intestinal microvilli (Iv). Note that the *Giardi*a trophozoite is free from bacterial endosymbionts and the surrounding Paneth cells (Pc) appear inactive and not stimulated. Inactive Goblet cells (Gc). (× 6600.)

https://doi.org/10.1017/S0031182004006547 Published online by Cambridge University Press

The authors would like to thank Dr Samir A. Zaki, Associate Professor of histology, Faculty of Medicine, Zagazig University, Egypt, for his kind assistance in this work.

REFERENCES

ALEY, S. B., ZIMMERMAN, M., HETSKO, M., SELSTED, M. E. & GILLIN, F. D. (1994). Killing of *Giardia lamblia* by cryptdins and cationic neutrophils peptides. *Infection and Immunity* **62**, 5397–5403.

AYABE, T., SATCHELL, D. P., WILLSON, C. L., PARKS, W. C., SELSTED, M. E. & OUELLETTE, A. J. (2000). Secretion of microbicidal α-defensins by intestinal Paneth cells in response to bacteria. *Nature, Immunology* **1**, 113–138.

DE-SOUZA, W. & MOTTA, M. C. (1999). Endosymbiosis in protozoa of *Trypanosomatidae* family. *FEMS Microbiology Letters* **173**, 1–8.

GLAUERT, A. M. (1980). *Fixation, Dehydration and Embedding of Biological Specimens*. 3rd Edn. North-Holland Publishing, Amsterdam.

JONES, D. E. & BEVINS, C. L. (1992). Paneth cells in the human small intestine express an antimicrobial peptide gene. *Journal of Biological Chemistry* **267**, 23216–23225.

MARTIN, E., GANZ, T. & LEHRER, R. I. (1995). Defensins and other endogenous peptide antibiotics of vertebrates. *Journal of Leukocyte Biology* **58**, 128–136.

MENG, T. C., HETSKO, M. L. & GILLIN, F. D. (1993). Antigenic switching of TSA 417, a trophozoite surface protein, following of the life cycle of *Giardia lamblia*. *Infection and Immunity* **61**, 5394–5397.

MEYER, E. A. (1990). Taxonomy and nomenclature. In *Giardiasis* (ed. Meyer, E. A.), pp. 51–60. Elsevier, Amsterdam.

NEMANIC, P. C., OWEN, R. L., STEVENS, D. P. & MULLER, J. C. (1979). Ultrastructural observations on giardiasis in a mouse model. II. Endosymbiosis and organelle distribution in *Giardia muris* and *Giardia lamblia*. *Journal of Infectious Diseases* **140**, 222–228.

OUELLETTE, A. J. & SELSTED, M. E. (1996). Paneth cell defensins: Endogenous peptide components of intestinal host defense. *FASEB Journal* **10**, 1280–1289.

ROBERTS-THOMSON, I. C., STEVENS, D. P. & MAHMMOUD, A. A. F. (1976). *Giardia* in mouse: an animal model. *Gastroenterology* **71**, 57.

SELSTED, M. E., MILLER, S. I., HENSCHEN, A. H. & OUELLETTE, A. J. (1992). Enteric defensins: antibiotic peptide component of intestinal host defense. *Journal of Cell Biology* **118**, 929–939.