Chapter 14

Organ Culture as a Model System for Studies on Enterotoxin Interactions with the Intestinal Epithelium

Ulver Spangsberg Lorenzen, Gert H. Hansen, and E. Michael Danielsen

Abstract

Studies on bacterial enterotoxin-epithelium interactions require model systems capable of mimicking the events occurring at the molecular and cellular levels during intoxication. In this chapter, we describe organ culture as an often neglected alternative to whole-animal experiments or enterocyte-like cell lines. Like cell culture, organ culture is versatile and suitable for studying rapidly occurring events, such as enterotoxin binding and uptake. In addition, it is advantageous in offering an epithelium with more authentic permeability/barrier properties than any cell line, as well as a subepithelial lamina propria, harboring the immune cells of the gut mucosa.

Key words Organ culture, Enterotoxins, Brush border, Enterocytes, Small intestine, Mucosal explants

1 Introduction

Enterotoxins are proteins produced by invasive pathogenic microorganisms and secreted in the intestinal lumen to weaken the defense of the host organism with the aim to facilitate colonization. Infections with enterotoxin-producing bacteria typically cause secretory diarrhea, vomiting, and in severe cases toxic shock and death, and collectively they are responsible for a huge worldwide morbidity and mortality [1]. Whereas some enterotoxins, for instance those of *V. cholerae* and enterotoxigenic *E. coli*, directly target the absorptive epithelium [2], others, like those of *S. aureus*, primarily target the immune cells in the underlying lamina propria by acting as "superantigens" [3]. By tradition, studies on enterotoxin-epithelium interactions most often rely either on animal experiments or epithelial cell cultures, but in this chapter we describe organ culture as an alternative model system to be considered. Introduced several decades ago for human biopsies by Browning and Trier [4], organ culture may keep intestinal tissue viable for periods up to 24 h, permitting studies on metabolism,

cell proliferation, and nutrient absorption. Applying the technique to porcine mucosal explants [5], we have used organ culture for a number of years in studies of a great many processes related to various aspects of gut mucosal biology. Feeling that organ culture today is being somewhat neglected by experimental researchers in the field of gastroenterology, and based on our own experience, we wish to present this technique as an attractive candidate model system for studies on enterotoxin-epithelium interactions.

Like cell culture, organ culture is versatile and is suitable for short-term exposures (on the minute scale), when early events in the enterotoxin-epithelium interaction are under investigation. In addition, by comparison with cell culture, organ culture offers two more advantages:

1. A native epithelium displaying authentic permeability/barrier properties: Though polarized, enterocyte-like cell lines (for instance Caco-2 and HT-29) do not possess an apical, fully developed brush border including a subapical, filamentous terminal web and a full complement of digestive enzymes and nutrient transporters at the cell surface.

2. An in vivo-like epithelial organization possessing an underlying lamina propria containing cells of the immune system in the gut: This makes it feasible to study for instance synthesis, epithelial transcytosis, and apical secretion of locally produced immunoglobulins [6, 7].

We have used organ culture of porcine jejunal mucosal explants as a model system to study binding and uptake of enterotoxins from *V. cholerae* and *S. aureus* [8, 9]. In these works, we were able to show that cholera toxin subunit B binds to lipid raft microdomains at the enterocyte brush border and is taken up by an induced clathrin-dependent mechanism (Fig. 1) [9]. In contrast, the two staphylococcal enterotoxins, SEA and SAB, only associated poorly with lipid rafts but were taken up into a distinct population of early endosomes in the terminal web region (TWEEs), from where they engaged in transcytosis to reach their target cells in the subepithelial lamina propria (Fig. 2) [8].

Fig. 1 (continued) whereas in others, both enterotoxins (*yellow arrows*) were taken up into the same endosomes. The labeling pattern suggests that although CTB and HLT bind to a common receptor, ganglioside GM_1, at the cell surface, they exhibit some variation in their interaction profile with the intestinal epithelium. Nuclei were visualized by DAPI in the merged image. Bar: 10 μm
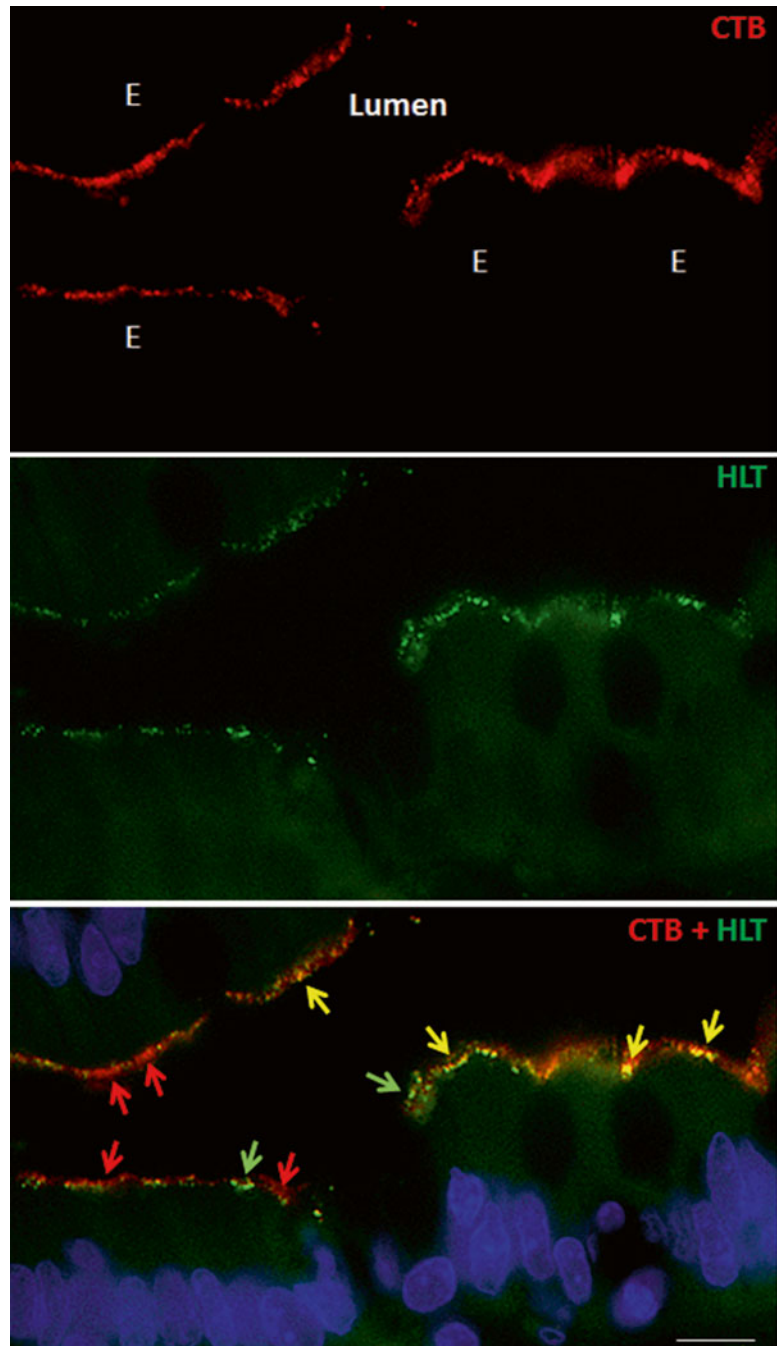
Fig. 1 Binding and uptake of CTB (cholera toxin B subunit) and HLT (heat-labile toxin of enterotoxigenic *E. coli*) in organ-cultured porcine jejunal explants. The mucosal explants were cultured for 1 h at 37 °C in RPMI medium in the presence of 10 μg/ml Alexa 594-conjugated CTB and 10 μg/ml FITC-conjugated HLT. Images of cryosections show binding of both enterotoxins to the luminal brush border of enterocytes (E) along the villi and uptake into subapical punctae. In some cells, only CTB (*red arrows*) or HLT (*green arrows*) was internalized,
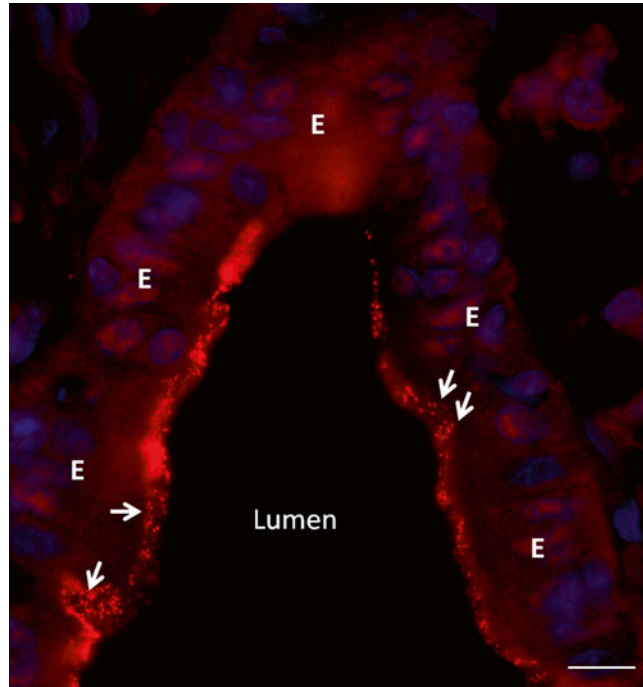
Fig. 2 Binding and uptake of SEB (*S. aureus* enterotoxin B) in organ-cultured porcine jejunal explants. Organ culture in the presence of 10 µg/ml FITC-SEB was performed as described in the legend to Fig. 1. After culture the explants were quickly rinsed in fresh medium and fixed overnight in 4 % paraformaldehyde in PBS. Cryosections of the explants were cut and immunolabeled with a mouse monoclonal antibody to fluorescein (Invitrogen), followed by labeling with Alexa 594-conjugated rabbit secondary antibodies (Invitrogen). Nuclei were visualized by DAPI. The image shows binding and uptake of SEB along the brush border of the enterocytes (E). Internalized SEB is seen as distinct punctae which in some cells (marked by *arrows*) had penetrated deep into the cytoplasm. Bar: 10 µm

2 Materials

1. Scalpels, scissors, and tweezers for excision and trimming of mucosal explants.

2. A suitable culture medium, for instance Roswell Park Memorial Institute (RPMI) medium (*see* **Note 1**).

3. 10 % (v/v) fetal calf serum, 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin are recommended to be added to the culture medium for longer periods of culture (>5 h).

4. "Falcon" dishes for organ culture (Fig. 3) are in vitro fertilization dishes in polystyrene, 60×15 mm style with a center well, and a triangular grid of stainless steel (Cat. # 353653, Beckton Dickinson, www.bdbiosciences.com).
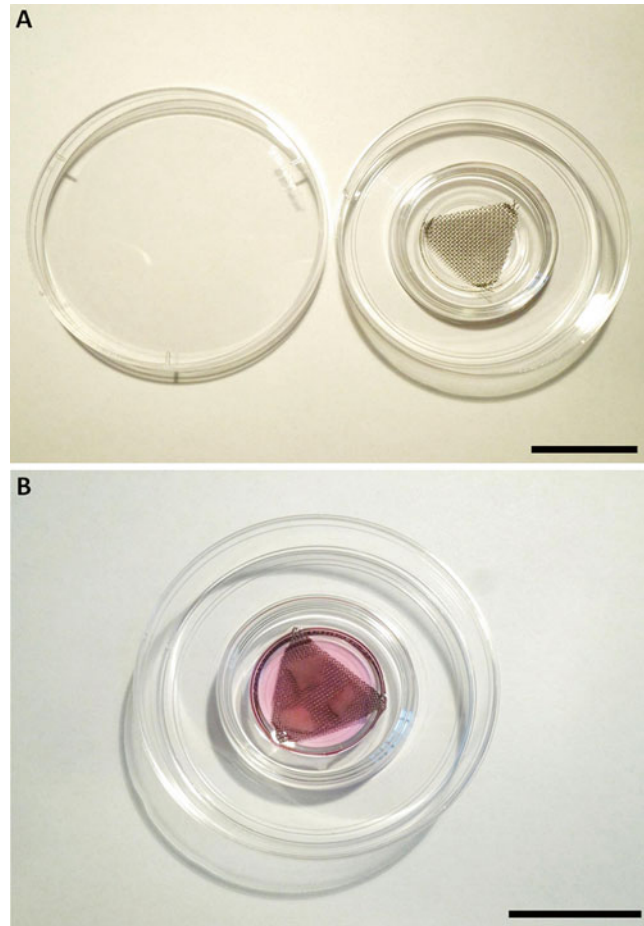
5. A suitable incubator.

Fig. 3 A Falcon dish with lid for organ culture of intestinal explants. (**a**) Culture dish with a rimmed central well 2 cm in diameter in which a triangular grid of stainless steel is mounted. (**b**) Three mucosal explants placed on the metal grid villus side upwards and 1 ml of medium added to the central well. This volume ensures that the explants are just submerged in the medium. The medium should be replaced once for every 1–2 h of culture. Bars: 2 cm

3   Methods

3.1   Obtaining Intestinal Segments from a Donor Animal (See Note 2)

1. In a licensed facility for animal experimentation, a donor pig is placed at an operating table, maintained under anaesthesia, and connected to a respirator (*see* **Note 3**).

2. An abdominal incision is performed to expose the small intestine and to excise small segments (~10 cm) of the desired parts of the intestine (*see* **Note 4**).

3. The excised intestinal segments are cut open longitudinally to expose the mucosal surface before quickly being immersed in ice-cold RPMI medium.

3.2 Organ Culture Setup

1. Without delay and within 5–10 min, the intestinal segment is placed villus side up in a Petri dish. Medium is quickly added to the dish to keep the tissue moist at all times.

2. Using tweezers and a scalpel, small (~1 cm^2) pieces of mucosa are removed from the underlying serosal/muscularis layers, taking care not to damage the villus surface in the process (*see* **Note 5**).

3. When sufficient free mucosa has been obtained, small uniform explants (~2–3 mm squares) are excised with the scalpel and carefully placed villus side upwards on the metal grids. Up to three explants of this size can be mounted on each grid.

4. When mounted, excess medium is soaked off from the grid by placing it briefly atop a sheet of tissue paper.

5. The grid is placed in the central well of a culture dish and 1 ml of medium is added. This volume will ensure that the explants are immersed just beneath the surface of the medium (*see* **Note 6**).

6. Place the culture dish in the incubator at 37 °C. We routinely culture the mucosal explants for 15 min to allow for acclimatization before starting any experiment such as addition of enterotoxins to the culture dish (*see* **Note 7**).

7. Change the medium every hour. For longer periods of culture (>5 h), it is recommended to gas the cultured tissue with O_2/CO_2 (19:1) for short intervals (3–20 min) every 5 h [4, 5].

8. For termination of organ culture, the grid with the mucosal explants is removed from the culture dish with tweezers and washed once very gently with fresh medium by use of a Pasteur pipette.

9. Excess medium is soaked off as described in **step 4**. The further procedure depends upon which type of analysis will be performed (*see* **Note 8**).

4 Notes

1. The original medium recommended for organ culture was Trowell's T-8 medium [4], but to our knowledge this medium is no longer commercially available.

2. The protocol described here relates to our work using porcine small intestine. Organ culture of small intestine from smaller animals such as rabbit [10], guinea pig [11], rat [12], and mouse [13] has been reported by other investigators.

3. It is our experience that the tissue viability of intestinal mucosal explants crucially depends upon a maintained blood circulation in the donor animal at this step. Circulatory arrest prior to removal of the intestinal segment, even for a short period of time, is harmful to the integrity of the intestinal mucosa. For organ culture of intestine from smaller animals, such as mice, the intestine is removed immediately after sacrifice of the animal.

4. All animal handling steps must be performed by licensed staff and subject to national laws and regulations governing animal experimentation.

5. This maneuver can be tricky to perform and some practice may be required. Cutting/scraping with the scalpel at a fixed angle to the tissue with one hand, whilst holding the tissue firmly in place with the tweezers in the other hand, is one way to obtain satisfactory results. For organ culture of intestine from mice, we omit this step and instead excise small pieces of whole intestine to be placed directly in culture dishes.

6. Overall, the procedure described in points 2–4 should be completed within ~15 min to obtain a good viability during the subsequent organ culture. This sets a limit as to how many culture dishes that can be set up by one person in a single experiment; 10–12 culture dishes is a feasible number.

7. When adding small volumes of agents to the culture medium, the tip of the pipette should not be pointed directly at the tissue. After addition, the medium is mixed by repeated, gentle use of a 200 µl pipette, still taking care not to point it at the tissue.

8. If biochemical analyses are to be performed, the tissue is quickly transferred to an Eppendorf tube and frozen until further processing. For microscopy, the explants are directly immersed in a suitable fixative, for instance 4 % paraformaldehyde in PBS, pH 7.4, and fixed at 4 °C for 2 h or overnight. After fixation, the explants are kept in 1 % paraformaldehyde in PBS, pH 7.4 at 4 °C until further processing.

Acknowledgements

References

1. Guerrant RL, Oria R, Bushen OY, Patrick PD, Houpt E, Lima AA (2005) Global impact of diarrheal diseases that are sampled by travelers: the rest of the hippopotamus. Clin Infect Dis 41(Suppl 8):S524–S530

2. Glenn GM, Francis DH, Danielsen EM (2009) Toxin-mediated effects on the innate mucosal defenses: implications for enteric vaccines. Infect Immun 77:5206–5215

3. Choi YW, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J (1989) Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. Proc Natl Acad Sci U S A 86:8941–8945

4. Browning TH, Trier JS (1969) Organ culture of mucosal biopsies of human small intestine. J Clin Invest 48:1423–1432

5. Danielsen EM, Sjostrom H, Noren O, Bro B, Dabelsteen E (1982) Biosynthesis of intestinal microvillar proteins. Characterization of intestinal explants in organ culture and evidence for the existence of pro-forms of the microvillar enzymes. Biochem J 202:647–654

6. Hansen GH, Niels-Christiansen LL, Immerdal L, Hunziker W, Kenny AJ, Danielsen EM (1999) Transcytosis of immunoglobulin A in the mouse enterocyte occurs through glycolipid raft- and rab17-containing compartments. Gastroenterology 116:610–622

7. Hansen GH, Niels-Christiansen LL, Immerdal L, Danielsen EM (2006) Antibodies in the small intestine: mucosal synthesis and deposition of anti-glycosyl IgA, IgM, and IgG in the enterocyte brush border. Am. J. Physiol Gastrointest. Liver Physiol 291:G82–G90

8. Danielsen EM, Hansen GH, Karlsdottir E (2013) Staphylococcus aureus enterotoxins A- and B: binding to the enterocyte brush border and uptake by perturbation of the apical endocytic membrane traffic. Histochem Cell Biol 139:513–524

9. Hansen GH, Dalskov SM, Rasmussen CR, Immerdal L, Niels-Christiansen LL, Danielsen EM (2005) Cholera Toxin Entry into Pig Enterocytes Occurs via a Lipid Raft- and Clathrin-Dependent Mechanism. Biochemistry 44:873–882

10. Kagnoff MF, Donaldson RM Jr, Trier JS (1972) Organ culture of rabbit small intestine: prolonged in vitro steady state protein synthesis and secretion and secretory IgA secretion. Gastroenterology 63:541–551

11. Kedinger M, Haffen K, Hugon JS (1974) Organ culture of adult guinea-pig intestine. I. Ultrastructural aspect after 24 and 48 hours of culture. ZZellforsch Mikrosk Anat 147:169–181

12. Shields HM, Yedlin ST, Bair FA, Goodwin CL, Alpers DH (1979) Successful maintenance of suckling rat ileum in organ culture. Am J Anat 155:375–389

13. Berteloot A, Chabot JG, Menard D, Hugon JS (1979) Organ culture of adult mouse intestine. III. Behavior of the proteins, DNA content and brush border membrane enzymatic activities. In Vitro 15:294–299