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Rat, ovine and bovine Peyer's patches mounted in horizontal diffusion chambers display sampling function

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Abstract

Freshly excised rat, ovine and bovine ileal Peyer's patch (PP) and non-Peyer's patch tissues (NPP) were mounted in modified horizontal polyethylene diffusion chambers with a range of window areas. Rat tissue was initially used to establish that barrier function and histology were maintained for up to 60 min. Horse-radish peroxidase (HRP) fluxes and *S. Typhimurium* adherence and invasion were significantly higher in rat PP over NPP. Particle uptake was shown to be a rapid, energy-, time-, and size-dependent process, occurring more readily in PP than NPP tissue in each species. In a kinetic analysis, particles were localized initially in the follicle-associated epithelium and then in the dome region. For NPP uptake, particles were initially localized to villous epithelium, and were then detected in the crypts and lamina propria. Electrophysiological parameters including pharmacologically-stimulated inward short-circuit current responses were determined in isolated PP and NPP from each species mounted under identical conditions in Ussing chambers. In conclusion, comparative functional and histological characteristics of PP from several species were demonstrated in horizontal diffusion chambers. Horizontal diffusion chambers are therefore a useful *in vitro* model in which a range of functions including transport of particulate formulations by PP may be examined.

Keywords: Peyer's patches; M cells; Particle absorption; Bacterial adhesion; Ussing chamber

1. Introduction

Intestinal Peyer's patches (PP) serve a role in the sensory immune system and are potentially important targets for oral peptide and vaccine delivery. PP M cells appear to translocate a range of particulates more avidly than villous epithelia [1,2], but there is considerable debate over the scale and the kinetics of these processes [3–5] as well as on the potential impact of an apparent reduced level of PP-overlying mucus on particle M cell binding in vivo [6]. M cells also appear capable of selective uptake of pathogens including viruses [7], bacteria [8] and prions [9]. The sampling portal function of M cells aids in the development of mucosal immune responses to challenge, while at the same time maintaining tolerance to common orally-administered antigens. M cell research is therefore evolving along two related strands, one in understanding the targeting strategies of pathogens and the other in formulating M cell targeted oral vaccine formulations, both underpinned by research into how these unique cells differentiate.

M cells within the murine follicle-associated epithelium (FAE) overlying the lymphocyte-rich dome region have been successfully targeted with stable liposomes [10], poly-(D,L-lactide)-co-glycolide (PLG) microparticles [11] and latex microparticles bearing conjugated lectins specific for the α-L-fucose receptor [12]. In addition, the recent application of human M-like cell models designed by converting Caco-2 to an M cell phenotype by co-culturing with lymphocytes has displayed many expected features of human M cells [13–15]. These include greater association of bacteria, higher rates of particle uptake and differential expression of apical membrane markers including alkaline phosphatase and β1 integrin.

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In this study, we re-examine the PP phenotype in fresh intestinal tissue since M-like cell culture reductionist models have obvious limitations. In order to confirm its applicability we used tissue from three different species. Classical Ussing chamber configurations, in which mucosal sheets are mounted vertically, have been used to measure electrophysiological parameters of rabbit PP [16] and to determine flux rates of large proteins including horse-radish peroxidase (HRP) across porcine PP [17]. The difficulties of measuring particle uptake in Ussing chambers include artifacts relating to non-specific binding to system components. In addition, an uplift-carbogen gas system provides aeration and mixing in Ussing chambers, but this is sub-optimal for allowing significant particle contact time with the epithelium. Recent data suggests that there may be an issue of compromised viability of intestinal tissue unless mucosae are mounted in chambers within a very short time after loss of blood supply [18].

Horizontal chamber designs have been used primarily as tools to investigate drug transport across isolated airway epithelia under conditions simulating the air–mucosa interface [e.g. 19]. The rationale was that very small volumes of bathing medium containing high concentrations of drug formulations in solution, gel or particulate formats could be added to mucosal side of the tissue. We have developed a simple, economical and disposable horizontal polyethylene chamber system that can be modified for intestinal tissue derived from both rodents and larger animals. Thus, the aims of this study are two-fold. First, we attempt to show that a new horizontal disposable chamber is appropriate for measuring several functional parameters of isolated intestinal tissue and secondly, we attempt to compare microparticle uptake by isolated PP from both ruminants and non-ruminants.

2. Materials and methods

2.1. Materials

Adult male Wistar rats (300–350 g, $n=91$) were obtained from the Biomedical Facility, University College Dublin, Ireland. 3–5 month old lambs ($n=7$) and calves ($n=5$) were reared at the Central Veterinary Research Laboratory, Abbotstown, Ireland. All animals were unfasted. Eppendorf polyethylene tubes were obtained from Starstedt, Ireland. Horse-radish peroxidase (HRP, Type VI, Molecular Weight 40 kD) and neutral red were purchased from Sigma–Aldrich, UK. Fluorescein-5-isothiocyanate (FITC)-labeled carboxylated latex particles were obtained from Molecular Probes, Oregon, USA.

2.2. Intestinal tissue preparation

Rats were sacrificed by cervical dislocation, while lambs and calves were initially anaesthetised with a sub-lethal dose of intravenously-administered pentobarbital (30 mg/kg). Intestinal tissue was exposed in each species via a mid-ventral abdominal incision. PP from each species were obtained from the distal ileum and NPP were obtained from the proximal ileum. Segments were excised and immediately immersed in freshly prepared oxygenated Krebs–Henseleit solution (KH) at room temperature. Following intestinal excision from lambs and calves, the animals were euthanased with a lethal dose of pentobarbital. The rationale for the two stage anaesthetic protocol for lambs and calves was that intestinal tissue in large animals is especially susceptible to hypoxia [20]. Consequently, it was important to maintain intestinal tissue perfusion *in vivo* until final excision. All procedures in the study adhered to the Principles of the Laboratory Animal Care (NIH Publication # 85–23, revised in 1985). Large animal studies were carried out in accordance with Irish Department of Health and Children Animal Licence Number, B100/3568.

An average of four PP, each of an approximate diameter of 0.2 cm, were located in the small intestine of each rat. Ovine and bovine ileal PP were not recognisable macroscopically either by use of a light source or by palpation, even though they comprised a significant portion of the distal ileum as indicated by microscopic examination. Sections designated as PP were confirmed histologically by haemotoxylin and eosin (H and E) staining at the end of each experiment. Intestinal tissues from each species were opened along the mesenteric border and rinsed with KH to remove intestinal contents. For particle uptake and histological studies, sub-mucosal muscle was left *in situ* in order to reduce dissection time. For HRP transepithelial flux studies, the underlying muscle was rapidly removed from mucosae in order to facilitate passage of HRP to the sampling receiver compartment. For these experiments, tissue was pinned mucosal-side down on a corkboard and muscle was removed by blunt dissection.

2.3. Horizontal diffusion chambers

Horizontal diffusion chambers were gassed with 95% O_2:5% CO_2 on both sides of the tissues and a heated glass water jacket maintained temperatures of 37.5 °C. The chambers comprised two different Eppendorf tubes. Internal window areas of 0.4 cm^2 (blue tip) and 1.0 cm^2 (clear tip) were used for rat and large animal mucosae respectively. Mucosae were mounted between two tapered, fitted halves of the chambers within 15 min of death (Fig. 1). Because no pins or thread were used and the tapered fit was locked at a set distance, edge damage was minimised. The apical compartments contained volumes of either 1 mL or 4 mL KH for rat and in large animal tissue respectively. The basolateral compartments contained 10 mL KH for both rat and large animal tissue. In this constraint, there were no chemical, osmotic, hydraulic or hydrostatic gradients present.

2.4. Morphology and barrier evaluation

Structural integrity was evaluated by histological examination of PP and NPP rat tissue mounted for selected time periods up to 120 min. Following incubation, tissues were collected in formalin and fixed overnight. Three sections from each formalin-fixed block were collected on glass slides and examined by light microscopy. Computer assisted morphometric measurements were analyzed using a color video camera (JVC 3CCD FY-F55B) attached to a microscope (Nikon™ LabPhoto-2) linked to an image analysis program, Image-Pro™ Plus® software version