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Transport of nanoparticles across an in vitro model of the human intestinal follicle associated epithelium

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Abstract

An in vitro model of the human follicle associated epithelium (FAE) was characterized and the influence of nanoparticle properties on the transcellular transport across the in vitro model was investigated. The model was established by co-culturing Caco-2 and Raji cells, with Caco-2 cells alone as control. The conversion of Caco-2 cells to follicle associated epithelium (FAE) like cells was monitored by following the surface expression of β1-integrins (immunofluorescence) and nanoparticle transport (flow cytometry). The influence of the nanoparticle concentration at the apical side, temperature, size and surface properties of nanoparticles on transport was evaluated, as well as the influence of transport conditions. The conversion of Caco-2 cells into FAE-like cells occurred. The transport was concentration, temperature and size-dependent. Aminated nanoparticles were more efficiently transported than carboxylated nanoparticles, suggesting a role of nanoparticle surface functional groups and hydrophobicity, possibly leading to a different pattern of protein adsorption at their surface. In conclusion, this in vitro model is a promising tool to study the role of M cells in transintestinal nanoparticle transport, as well as to evaluate new drug delivery systems.

Keywords: M cells; Caco-2 cells; In vitro model; Oral delivery; Nanoparticles

1. Introduction

Drug delivery by the oral route is considered as the preferred route of administration, due to its convenience. It is user-friendly and reduces the risk of infection, as well as the pain for the patient, and possible contamination of the medical personnel. The intestinal epithelial barrier consists of a cell monolayer, predominantly composed of enterocytes interspersed by mucus-secreting goblet cells, that generally constitutes effective barriers, and prevents the uptake of microorganisms and other particles. Scattered throughout the gastrointestinal mucosa, the organized mucosa associated lymphoid tissues (O-MALT) is found (Clark et al., 2001b). O-MALT consists of lymphoid follicles arranged either singly or as clusters to form distinct structures, such as the Peyer's patches, situated immediately below the intestinal epithelial cell monolayer. These structures are separated from the lumen by the follicle associated epithelium (FAE), which differs from the normal intestinal epithelium in that it contains specialized epithelial M cells with the capacity to transport particulate matters, such as bacteria and viruses. These particular cells are mainly found in the FAE, although recently M cells have been identified in the villous epithelium and demonstrated to develop

Abbreviations: FAE, follicle associated epithelium; TEER, transepithelial electrical resistance; O-MALT, organized mucosa associated lymphoid tissues

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without the influence of the O-MALT structures (Jang et al., 2004).

The M cells are specialized for antigen sampling, but they are also exploited as a route of host invasion by many pathogens (Gebert et al., 1996; Kraehenbuhl and Neutra, 2000). Furthermore, M cells represent a potential portal for oral delivery of peptides and for mucosal vaccination, since they possess a high transcytotic capacity and are able to transport a broad range of materials, including nanoparticles (Clark et al., 2000; Frey and Neutra, 1997). However, despite the advantages of the oral route, most peptide and protein drugs as well as peptidomimetics available today are administered parenterally rather than orally. Their possible gastrointestinal degradation by digestive enzymes (Chen and Langer, 1998) and their very poor intestinal absorption (except for most peptides with no more than three or four amino acids) lead to a low oral bioavailability of such molecules. Because of the high potential and promising field that therapeutic peptides and proteins represent, new oral formulations have to be developed to tackle these difficulties. One delivery strategy could be based on the encapsulation of peptides in particulate carriers (liposomes, nano- or microparticles). This would protect the peptides against chemical and enzymatic degradation and potentially also enhance the selective uptake of these particles by M cells (Clark et al., 2001b).

Numerous studies have been performed on M cells to understand the strategies used by pathogens to exploit this pathway and to use their transport abilities for the delivery of vaccines to the mucosal immune system (Clark et al., 2001b; Frey and Neutra, 1997; Hussain and Florence, 1996; Kraehenbuhl and Neutra, 2000; Neutra et al., 1999; Owen, 1999). However, the more precise role played by M cells in the immune response, as well as the mechanisms of particle uptake and transport, remains poorly understood. In addition, only few specific markers of human M cells, e.g. the sialyl Lewis A antigen and cathepsin E (Finzi et al., 1993; Giannasca et al., 1999; Wong et al., 2003) have been identified. However, these results have not been confirmed since (Wong et al., 2003) and it remains difficult to identify and localize human M cells, limiting the progress in this field. Moreover, in vivo studies are difficult to perform and not always relevant due to the high variability of proportion and phenotype of M cells among different species (Brayden and Baird, 2001; Jepson et al., 1996).

An in vitro model was proposed (Kerneis et al., 1997) based on a “mixed” co-culture system of Caco-2 cells on inverted inserts and isolated lymphocytes from mouse Peyer’s patches. To overcome the use of primary murine lymphocytes, a new cell culture system has been developed to mimic the human FAE (Gullberg et al., 2000). It is based on the co-culture of Caco-2 cells on normally oriented inserts and human Raji B lymphocytes. Since our final objective is to study the oral delivery of vaccines or drugs designated to humans, the most relevant system for our application was the one developed by Gullberg et al. (2000).

Working with these in vitro models, the influence of the particle size (Gullberg et al., 2000), the temperature and the duration of exposure (Caliot et al., 2000; Ouzilou et al., 2002) on particle transcytosis through M cells was demonstrated. The influence of particle charge and hydrophobicity on transcytosis across M cells was also studied in vivo. In two independent studies, particles with a relatively high hydrophobicity were found to be absorbed more readily into mouse or rat Peyer’s patches (Eldridge et al., 1990; Hillery and Florence, 1996). In a third study (Keegan et al., 2003), a lower uptake of negatively charged polystyrene particles compared to non-ionized ones was observed in rats.

In this study, we investigated the influence of the physicochemical properties of the nanoparticles on particle transport across the human in vitro model of FAE, based on a co-culture of Caco-2 cells and human Raji B lymphocytes, developed by Gullberg et al. (2000). The influence of the nanoparticle concentration, the duration of incubation and the temperature on the particular transport across these cells, as well as the presence or absence of serum during transport experiments was also investigated.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Human colon carcinoma Caco-2 line (clone 1), obtained from Dr. Maria Rescigno, University of Milano-Bicocca, Milano, Italy (Rescigno et al., 2001), from passage $x+6$ to $x+10$, and human Burkitt’s lymphoma Raji B line (American Type Culture Collection, Manassas, VA) from passage 102 to 104, unless stated, were used.

2.1.2. Cell culture media and chemicals

Dulbecco modified Eagle’s minimal essential medium (DMEM, 25 mM glucose), RPMI 1640 medium, heat inactivated fetal calf serum, non-essential amino acids, L-glutamine and penicillin-streptomycin (PEST) were purchased from Gibco™ Invitrogen Corporation (Carlsbad, CA). Trypsin-EDTA consisted in 2.5% (w/v) of trypsin (Gibco™) and 0.2% (w/v) EDTA (IGN, Aurora, OH) in PBS (Gibco™). Hank’s Balanced Salt Solution buffer (HBSS) 10× was obtained from Gibco™, Hepes, and sodium bicarbonate from Sigma (St. Louis, MO). Rhodamine-phalloidine was obtained from Molecular Probes (Eugene, OR). Citric acid, urea, CHAPS and Triton, were purchased from Sigma. The Iso Electric Focusing gel Pharmalyte 3-10, and dithiothreitol (DTT) were from Amersham Biosciences (Uppsala, Sweden). Pefabloc® SC was from Roche (Indianapolis, IN).

2.1.3. Cell culture reagents, nanoparticles and antibodies

Transwell® polycarbonate inserts (12 wells, pore diameter of 3 μm, polycarbonate) were purchased from Corning