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Uptake and transport of copolymer biodegradable microspheres by rabbit Peyer's patch M cells

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Abstract. In this study, we demonstrate the role of M cells in uptake of poly(D-L-lactic-co-glycolic acid) (PLGA) microspheres and transport into rabbit Peyer's patches. Microspheres 1 to 10 μm in diameter composed of 50:50 lactic acid:glycolic acid were instilled into intestinal segments containing jejunal or ileal Peyer's patches, and uptake by M cells was examined by electron microscopy. PLGA microspheres visualized as electron-lucent, spherical particles were taken up by M cells by pseudopod-like extensions of the M cell apical membrane and translocated to the pocket region containing mononuclear leukocytes within 60 min. These results indicate that PLGA microspheres can be directed to M cell apical surfaces for delivery to immunocompetent cells in gut-associated lymphoid tissues.

Key words: Peyer's patches – Microspheres – Epithelium – M cells – Rabbit

Introduction

Biodegradable microspheres composed of poly (D,L-lactic-co-glycolic acid) (PLGA) have been used to deliver antigens to mucosal surfaces because of their ability to protect antigens from digestion in the stomach and intestine. They slowly release antigens in a time-dependent manner and are safe for use in humans (O'Hagan et al. 1991; Nellore et al. 1992; Reid et al. 1993). The oral administration of PLGA microspheres results in localization to mucosal and systemic lymphoid organs. In mice, PLGA microspheres 1–10 μm in diameter are transported into and through Peyer's patches, and confer both systemic and mucosal immunity (Eldridge et al. 1991). However, the initial cellular events in the recognition and uptake of PLGA particles into Peyer's patches have not been established.

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M cells preferentially bind macromolecules, microorganisms, and particulates 10 nm to 10 μm in diameter and transport them to immunocompetent cells in underlying mucosal lymphoid tissues (Wolf et al. 1983; Neutra et al. 1987; Pappo and Ermak 1989; Eldridge et al. 1991). The M cell surface is distinguished from that of intestinal absorptive cells by short, widely spaced microvilli and an intracellular vesicular transport system which facilitate phagocytosis and transcellular movement of particulates (Bye et al. 1984; Owen and Ermak 1990; Neutra and Kraehenbuhl 1992). Examination of murine models for targeted delivery of microparticles has been limited by sampling difficulties because only about 1/10 of follicle epithelial cells are M cells. In contrast, the rabbit has been an important model for the study of transepithelial antigen transport because M cells constitute about 50% of follicle epithelial cells, M cell enclose numerous intraepithelial leukocytes, and the transport efficiency of M cells in the rabbit is higher than in rodents (Pappo et al. 1988; Pappo and Ermak 1989; Pappo 1989; Ermak et al. 1991). In both models, identification of microspheres at the ultrastructural level has been difficult because of solvent effects on microspheres during tissue processing, and because of the low frequency of sampling particulates within this size range at the thin section level. In this study, we identify PLGA microspheres at the ultrastructural level and establish the role of M cells in their uptake into Peyer's patches.

Materials and methods

PLGA microspheres were prepared by solvent extraction (Reid et al. 1993). PLGA polymer (50:50 lactic acid:glycolic acid, molecular weight 58000), obtained from Medisorb Technologies International (Cincinnati, Ohio, USA), was dissolved in acetonitrile and emulsified in heavy paraffin oil (180–190 saybolt viscosity). The emulsion was then pumped into heptane to extract the oil and acetonitrile. Microspheres were recovered by centrifugation, washed with heptane, vacuum dried, and stored at −20° C. Size distribution analysis of resuspended microspheres evaluated by light microscopy (Reid et al. 1993) revealed that most particles were in

the 1–10 μm range (% volume distribution: <5 μm: 54%; 5–10 μm: 36%; >10 μm: 10%; n=150). A sample of freeze-dried microspheres was embedded as a pellet directly into an Epon/Araldite epoxy resin mixture for identification of the particles in thin section. The pellet was examined at three levels to account for differences in sedimentation rates between small and large particles.

Five female New Zealand White rabbits, 2 kg in weight, were obtained from Millbrook farms (Amherst, Mass., USA), fasted overnight, anaesthetized with 50 mg/kg ketamine plus 5 mg/kg xylazine, and a laparotomy was performed. Intestinal segments containing Peyer's patches were ligated and instilled with 375 μl PLGA microspheres containing about 10^9 particles resuspended in 0.1 M phosphate-buffered saline (PBS), pH 7.4 (Pappo and Ermak 1989). A total of 4 PBS control loops and 6 PLGA loops were prepared, using the most distal ileal patch and sequential patches towards the jejunum. After 30 or 60 min, Peyer's patches were harvested, rinsed with RPMI 1640 medium supplemented with 10 mM HEPES, 2 mM L-glutamine, and 5% fetal bovine serum, and processed for electron microscopy by fixation in a solution of 2.5% glutaraldehyde, 2% formaldehyde, and 0.1 M cacodylate

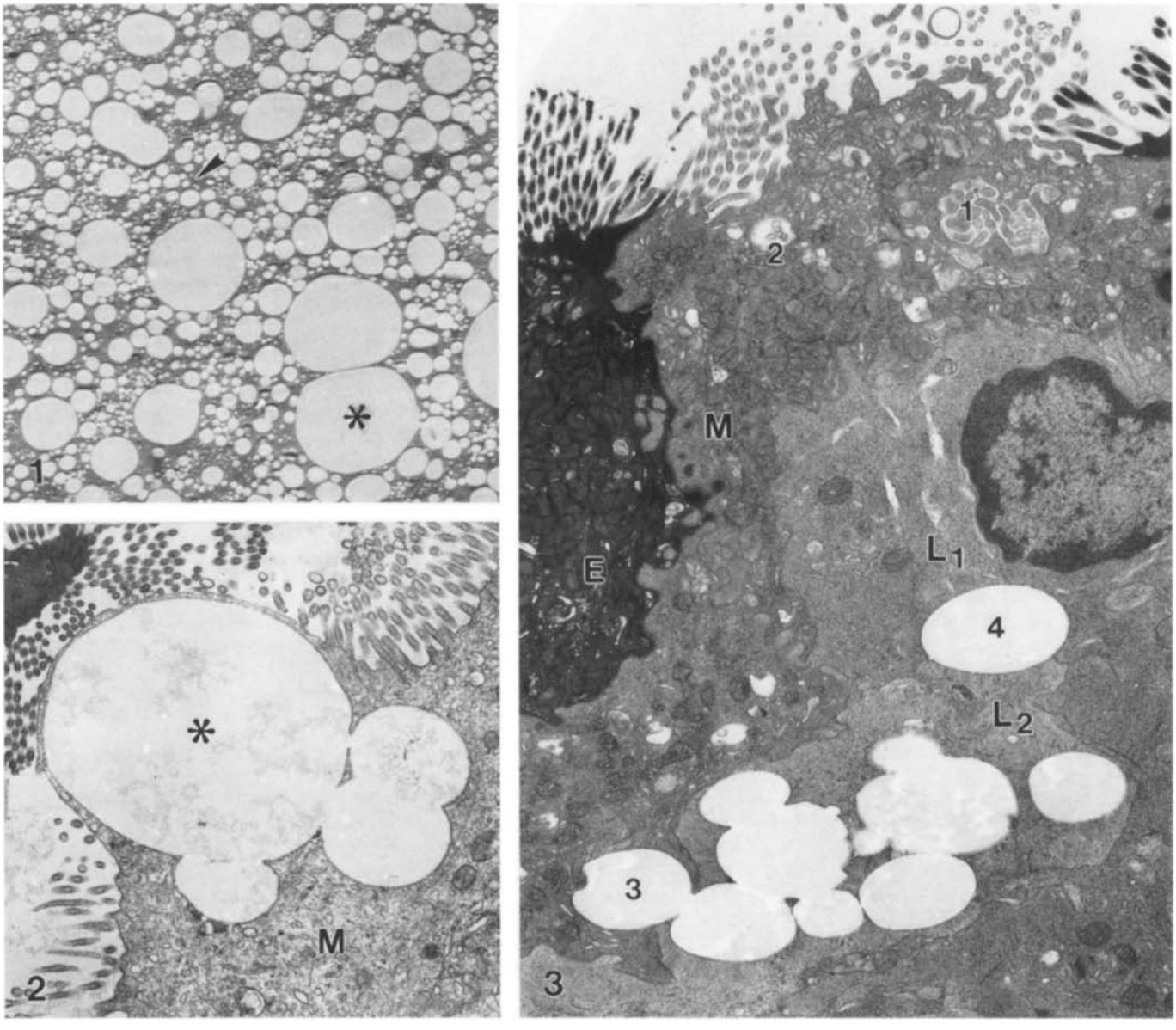

Fig. 1. Low power transmission electron micrograph of PLGA microspheres embedded in Epon/Araldite resin. Range of particle sizes from <1 μm (*arrowhead*) to 13 μm (*asterisk*) in diameter. ×1500

Fig. 2. Uptake of PLGA microspheres by M cell (*M*). Microspheres inoculated into rabbit's ligated intestinal loop containing Peyer's patch. Particles (1–4 μm in diameter) enclosed in M cell pseudopod; 4 μm microsphere (*asterisk*) enclosed by attenuated apical M cell process. ×11250

Fig. 3. Transepithelial transport of PLGA microspheres to M cell pocket. Particles (1–2.5 μm in diameter) transported by M cells to mononuclear leukocytes (*L*) enclosed in M cell (*M*) pocket. Structure *#1* represents invagination of M cell surface containing microvilli. Similar invaginations containing PLGA microspheres were also observed. Vesicle at *#2* represents polar section through microsphere positively identified in serial section of this field. Microsphere *#3* partially surrounded by M cell and in contact with mononuclear leukocyte, indicating exocytotic transport to extracellular pocket space. Microsphere *#4* translocated and positioned between two mononuclear leukocytes (*L1, L2*). Electron density of cytoplasm within enterocytes (*E*)>M cells>leukocytes. ×10000

buffer (pH 7.4). Tissues were post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.4), stained en bloc in uranyl acetate, and embedded in Epon/Araldite. Semi-thin 1-μm plastic sections stained with toluidine blue were examined by light microscopy, and thin sections were examined with a JEM-1200EX electron microscope. The entire surface of the follicle epithelium from 2–3 serial sections was scanned for the presence of PLGA particles.

Results and discussion

PLGA microspheres which were directly embedded in resin and thin sectioned appeared as clear, spherical particles from under 1.0 μm to over 10 μm in diameter [>500 particles sampled] (Fig. 1). The electron density of the particles was similar to that of the embedding medium. At both 30 and 60 min after instillation into intestinal loops, PLGA microspheres were identified in the apical portions of M cells, and within the epithelium, but could not be clearly identified in the lumen in association with M cell microvilli. PLGA particles phagocytosed by M cells were enclosed by pseudopodia extending from the M cell surface (Fig. 2). Microspheres were present in all domes examined and appeared as single particles or in groups of several particles. However, greater than 50% of the M cell sections did not contain microspheres. Within the epithelium, microspheres localized to the pocket region which was infiltrated by mononuclear leukocytes (Fig. 3). PLGA particles in tissue sections ranged in size from 1–4 μm [n=120], which distinguished them from intracellular, electron-lucent vesicles up to 1 μm in diameter. Microspheres could be identified in semithin sections stained with toluidine blue after prior examination of serial electron-microscopic images. The uptake of micrometer-sized PLGA particles by M cells appeared similar to uptake of bacteria in that direct contact between the M-cell apical membrane and the microsphere was followed by intracellular transport (Owen and Ermak 1990). No microspheres were observed within enterocytes of intestinal villi.

Uptake and transport of PLGA microspheres by M cells was qualitatively similar to that of fluorescent polystyrene microspheres (0.75–1.0 μm in diameter) which were transported at roughly 2 μm per min through the follicle epithelium into the dome (Pappo and Ermak 1989; Pappo et al. 1991). However, PLGA particles were more easily identified in thin sections because of their broader range of sizes. Both PLGA and polystyrene particles suffer from solvent extraction effects making identification difficult when dependent on inherent structural features. The frequency of observation in thin section (800 nm) is almost 100 times less than in frozen section (7 μm) because of sampling effects (Elias and Hyde 1983). The frequency of observation is also influenced by the size of particles and by the ability of M cells to take up different sized particles (Eldridge et al. 1991; Jani et al. 1990). M cells are capable of transporting particles from less than 100 nm to about 10 μm in diameter. Most PLGA particles observed in thin sectioned material in this study ranged between 1 and 4 μm in diameter, although the material instilled into loops included smaller and larger microspheres.

After passage across the epithelium, tracer particles have been shown to accumulate in macrophages in Peyer's patch domes and germinal centers, and enter the circulation (Joel et al. 1978; LeFevre et al. 1985; Eldridge et al. 1991). The basal lamina below the epithelium is porous allowing for the rapid transport of particles into the dome lymphoid compartment (McClugage et al. 1986; Pappo et al. 1988). In studies to determine subsequent distribution of biodegradable microspheres, it was found that particles <5 μm can be transported through Peyer's patches to peripheral lymphoid organs, whereas particles >5 μm remain within Peyer's patches (Jani et al. 1990; Eldridge et al. 1991). Thus, administration of particles which target immune cells within the Peyer's patches may be optimal for initiating IgA responses, whereas administration of particles which pass through patches may be optimal for the generation of systemic IgG responses.

It has been shown experimentally that modification of the surface of latex polystyrene particles can enhance M-cell mediated uptake (Pappo et al. 1991). The intimate contact between PLGA microspheres and M cells suggests that the surface chemistry of the PLGA microsphere may play a role in particulate recognition by M cells and could be modified to enhance uptake or to direct transport to mononuclear cells within lymphoid compartments.

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