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Uptake and translocation of fluorescent latex particles by rabbit Peyer's patch follicle epithelium: a quantitative model for M cell uptake

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SUMMARY

A quantitative, light microscopic morphometric model for uptake of particulates by Peyer's patch M cells was developed. Rabbit intestinal loops containing Peyer's patches were inoculated with fluorescent, non-degradable polystyrene microparticles (600–750 nm), and their localization in Peyer's patches was traced after varying time periods. The particles were localized sequentially at the FAE cell surface, spanning the entire width of FAE cells, and within the subepithelial dome as a function of time. The particles were associated with $5D9^+$ or $1D9^+$ M cells, but were not taken up or transported by villus epithelia. The kinetics suggested a synchronous wave of uptake and transepithelial transport. Quantitative analysis revealed a considerably greater uptake efficiency of polystyrene microspheres in comparison to other biological particles.

Keywords follicle-associated epithelium M cells Peyer's patches phagocytosis

INTRODUCTION

Gut-associated lymphoid tissues (GALT), represented by Peyer's patches, are primary sites of interaction between mucosal immunologic structures and antigens in the intestinal lumen. The GALT is composed of lymphoid follicles, interfollicular T cell-dependent areas, and a specialized follicle-associated epithelium (FAE) overlying lymphoid follicle domes (Owen & Jones, 1974; Abe & Ito, 1978; Sminia, Janse & Plesch 1983; Ermak & Owen, 1986). The FAE harbours M cells, differentiated for the uptake and transport of intestinal antigens. Peyer's patch M cells pinocytose soluble protein antigens (Bockman & Cooper, 1973; Owen, 1977), and represent specific sites for the uptake of viruses (Wolf *et al.*, 1981), bacteria (Owen *et al.*, 1986; Kohbata, Yokoyama & Yabuuchi, 1986), and protozoa (Marcial & Madara, 1986). Intestinal antigens endocytosed by M cells are rapidly transported to antigen presenting cells and lymphocytes in the FAE which are enclosed in the 'central hollow' or 'pocket region' of M cells (Owen, 1977; Owen *et al.*, 1986; Neutra *et al.*, 1987). However, soluble antigenic tracers can not be readily identified beyond the dome since these are rapidly diluted after transport (Owen, 1977), and particulate microbial antigens become unrecognizable once translocated into the dome, due to progressive catabolism and structural degradation (Owen, Allen & Stevens, 1981). Although M cells constitute specific sites of permeability to intestinal antigens, quantitative estimates of M cell-mediated uptake after in-vivo administration of antigens have not been established. The chronic feeding of insoluble latex microspheres to mice results in the deposition of large numbers of particles within Peyer's patches and mesenteric lymph nodes (LeFevre, Olivo & Joel, 1978), although the route of particle entry into the GALT has not been determined. Murine models of uptake are limited by the low frequency distribution of M cells, (Smith, Jarvis & King, 1980, Smith & Peacock, 1980), and by the low magnitude of uptake, as judged by ultrastructural analyses. In this study, we examined quantitatively the kinetics of uptake and transport of inert latex particles by rabbit Peyer's patch M cells, which comprise about 50% of the FAE cell population (Pappo, Steger & Owen, 1988). A light microscopic morphometric model was developed which allowed tracing of the events associated with M-cell-sampling of fluorescent polystyrene microspheres from the intestinal lumen into Peyer's patch domes.

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MATERIALS AND METHODS

Animals

Seven female New Zealand White rabbits, from 2·5 to 3 kg in weight, were purchased from Animals West (Soquel, CA) and Rabbitek (Modesto, CA).

Preparation of intestinal loops

The animals were fasted overnight, anaesthetized with Nembutal (40 mg/kg), and a laparotomy was performed. Intestinal

segments (1–3/animal) containing jejunal or ileal Peyer's patches were ligated and instilled through a 27 gauge needle with 250 μl of fluorescent polystyrene microspheres (600–750 nm diameter; Polysciences Inc., Warrington, PA) diluted to 10^9 particles/ml in 0·1 M phosphate-buffered saline (PBS), pH 7·4, or instilled with PBS only. In some experiments, alternating loops instilled with PBS only were constructed between loops containing latex particles. After 10, 30 or 90 min, the loops were harvested, and the Peyer's patches rinsed extensively with RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES buffer (M.A. Bioproducts, Walkersville, MD).

Tissue processing for immunofluorescence and morphometric analysis

Peyer's patch blocks, (2/time period/animal), 1 cm in length, were mounted in O.C.T. compound (Miles Scientific, Naperville, IL) and frozen in Freon 22 cooled to its freezing point by liquid nitrogen contained in a surrounding chamber (Ermak & Owen, 1986). Twenty-five to 50 cross sections per block were cut (7–10 μm) with a Hacker cryostat. Three separate Peyer's patch compartments were analysed for the presence or absence of particles: the FAE surface, defined as the outermost 10 μm of follicle epithelium, the remaining width of the FAE, represented by the region between the surface and the point of attachment to the basement membrane, and the subepithelial dome. The average length and width of the FAE overlying Peyer's patch domes were 1·5±0·1 mm, and 53±1 μm, respectively (mean ±standard error of the mean (s.e.m.) of nine Peyer's patches). Measurements were performed with a Zeiss Videoplan III image analyser. The number of particles enumerated within each compartment was standardized per unit length of epithelium. Cryosections were viewed in a Zeiss fluorescence microscope with filters for selective FITC excitation, and photographed with partial transmitted light to illuminate Peyer's patch tissue compartments.

Immunohistochemical staining of Peyer's patch FAE

The immunohistochemical localization of Peyer's patch M cells after in-vivo challenge with fluorescent microspheres was performed using monoclonal antibodies (MoAb) 5D9 (IgG2a,κ) and 1D9 (IgM,κ), which recognize rabbit M cells (Pappo, 1989). Briefly, the cryosections were incubated sequentially with hybridoma supernatants, biotinylated goat anti-mouse IgG or IgM (2·5 μg/ml), and avidin conjugated to biotinylated horseradish peroxidase (ABC; Vector Laboratories, Burlingame, CA). Reagents were applied to tissue sections for 30 min and the sections washed three times with PBS. Labelling sites were visualized with 0·05% diaminobenzidine tetrahydrochloride (Organon Teknika, Durham, NC) and 0·03% H_2O_2 in 0·1 M Tris HCl buffer at pH 7·3. Control cryosections were incubated with biotinylated secondary antibodies, or with isotype-matched MoAb of unrelated specificity.

RESULTS

Quantitative distribution of fluorescent latex particles, and kinetics of uptake

Whereas intraluminally administered fluorescent latex microspheres localized specifically in 45/45 of the Peyer's patch lymphoepithelial domes examined (Table 1), their concentration within each Peyer's patch compartment differed markedly as a function of time (Figs. 1a–c). In contrast, the epithelium and lamina propria of adjacent villi did not contain fluorescent latex particles at any time period examined (Fig. 1d).

Table 1. Frequency distribution of fluorescent latex microspheres in Peyer's patches after intraluminal in-vivo administration

Time after exposure (min)	Peyer's patch compartment		
	FAE surface	FAE	subepithelial dome
10	15/15*	9/15	0/15
30	15/15	15/15	11/15
90	15/15	15/15	15/15

* Data represent the number of Peyer's patch lymphoepithelial domes containing >10 particles per mm length/total number of lymphoepithelial domes examined.

After a 10 min exposure *in vivo*, 95% of the total microspheres observed in the Peyer's patches were localized at the FAE surface (Table 2). The fluorescent microspheres were often organized in clusters alternating every 10–15 μm with areas devoid of microspheres (Fig. 1a).

Thirty minutes post-introduction into ligated intestinal loops, the fraction of particles observed at the surface of the FAE decreased to 57±7% of the total microspheres residing in the Peyer's patches, whereas the proportion of particles spanning the entire width of FAE increased approximately eight-fold compared with the 10 min time period (Table 2). Aggregates of fluorescent particles were localized at discrete sites in the apical half of the FAE in a pattern which corresponded to the pocket region of M cells (Fig. 1e). At 30 min only a small proportion of the total particle count (3%) appeared within the subepithelial dome.

After 90 min latex particles populated the entire width of the FAE and penetrated approximately 120 μm into the dome lymphoid tissue (Fig. 1c). Nearly half of the total microspheres were found within the Peyer's patch dome compartment, while the proportion of particles at the FAE cell surface decreased to 17±5% (Table 1). The fraction of particles located within the FAE remained essentially unchanged. Focal accumulations of latex microspheres were seen within the dome compartment (Fig. 1c), suggesting an association with large cells, possibly dome macrophages.

As shown in the representative experiment in Table 2, the mean number of fluorescent particles found within Peyer's patch lymphoepithelial domes at each time interval ranged from 1800–2400/mm length. Whereas the average number of particles residing at the FAE surface was comparable at 10 and 30 min, the mean number of particles within the FAE itself increased substantially with advancing time (Table 2). At 90 min the number of particles found at the FAE surface decreased approximately four-fold, while the number of particles within the subepithelial dome increased markedly.

Localization of fluorescent latex particles in Peyer's patch FAE

The relationship between Peyer's patch M cells immunolabelled with MoAb 5B11 and 1D9 and the phagocytosed latex particles was examined. The MoAb labelled the basolateral pocket region

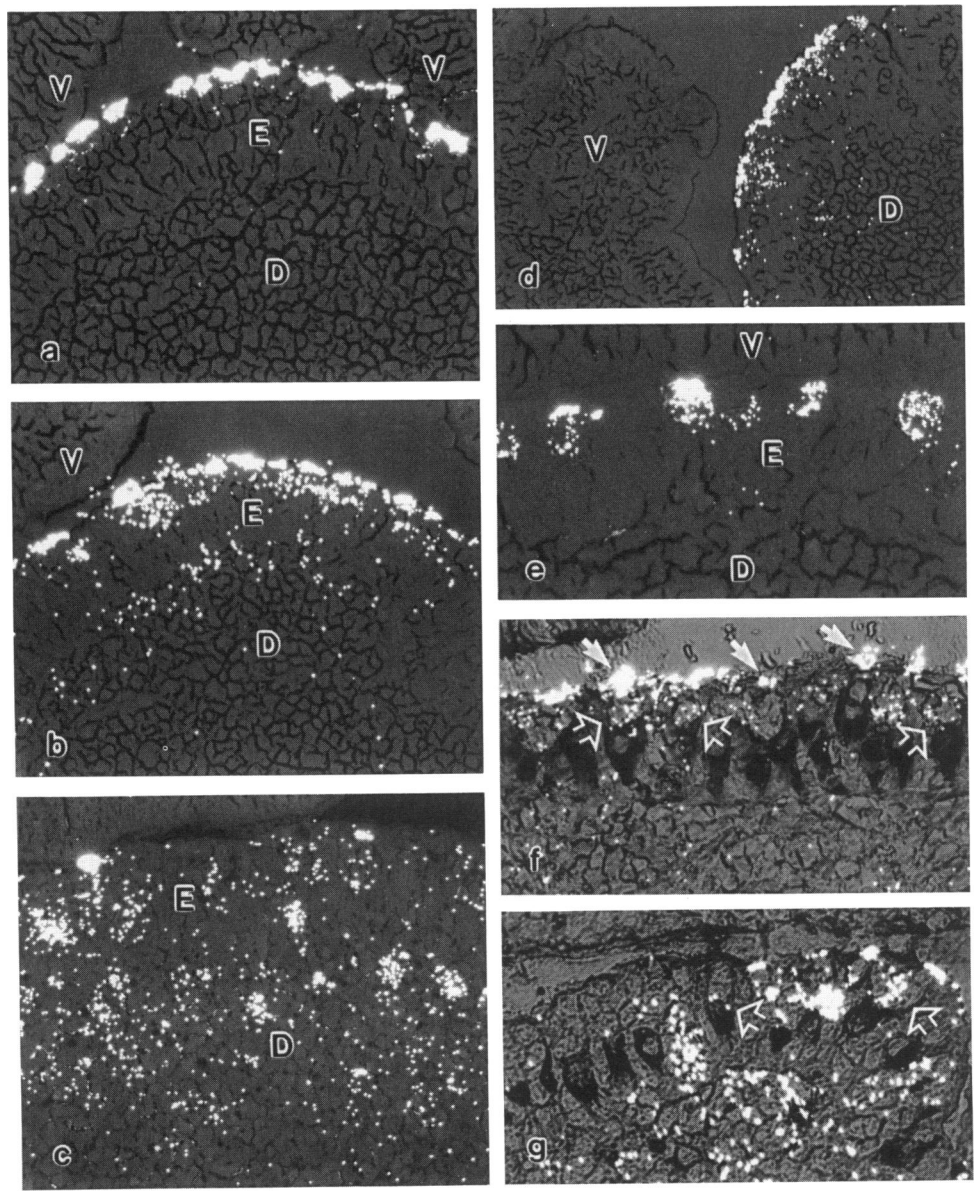

Fig. 1. Sequential uptake and transport of fluorescent polystyrene microspheres by rabbit Peyer's patch FAE cells, and their non-random localization at various time periods after inoculation into ligated intestinal loops containing Peyer's patches. At 10 min (a), virtually all particles are at the surface of the follicle epithelium (E). At 30 min (b) particles are primarily located at the surface and within the follicle epithelium (E). At 90 min (c), most particles are located within the subepithelial dome (D). Uptake of microspheres (d) by follicle epithelium (E) overlying Peyer's patch dome (D), but not by villus (V) epithelium (30 min specimen). Localization of microspheres (e) in discrete aggregations in apical regions of follicle epithelium (E) corresponding to the pocket region (30 min specimen). Association of microspheres with follicle epithelial M cells labelled with MoAb 5D9 (f) and 1D9 (g) after a 30 min exposure. Microspheres are located at the surface (arrows) and within pocket regions (open arrows) defined by labelled M cells. Original magnifications: (a–c) × 260; (d) × 160; (e–g) × 340.

of alternating M cells populating the FAE. The $5D9^+$ or $1D9^+$ M cell pocket region enclosed numerous fluorescent particles residing within the FAE (Figs. 1f–g). Similarly, the clusters of fluorescent latex particles localized at the FAE cell surface were aligned with $5D9^+$ or $1D9^+$ M cells (figs. 1f–g).

DISCUSSION

In the present study, we examined quantitative parameters of uptake and transepithelial transport by Peyer's patch M cells of luminal particulates. We found that fluorescent latex particles were internalized by M cells within 10 min of administration *in vivo*, and that an increasingly greater fraction of microspheres was specifically transported across FAE cells into Peyer's patch domes as a function of time. The rate of transport of latex particles in the 600–750 nm range, as used in this study, was similar to that previously observed with soluble tracer antigens (Bockman & Cooper, 1973; Owen, 1977), lectin-ferritin conju-

Table 2. Quantitative analysis of uptake and transport of fluorescent latex microspheres from the intestinal lumen into Peyer's patches

Time after exposure (min)	Percentage of total*			Particle count†		
	FAE surface	FAE	Subepithelial dome	FAE surface	FAE	Subepithelial dome
10	95±1	5±1	0	1746 (648–3943)	57 (26–121)	1 (0–2)
30	57±7	39±6	3±1	1686 (470–2668)	636 (142–939)	70 (54–88)
90	17±5	35±1	48±4	482 (49–885)	621 (125–1364)	732 (132–1759)

* Mean values ± s.e.m. of 15 lymphoepithelial domes from $n = 3$ rabbits.

† Mean number and (range) of particles/mm length from 15 Peyer's patch lymphoepithelial domes in one representative experiment.

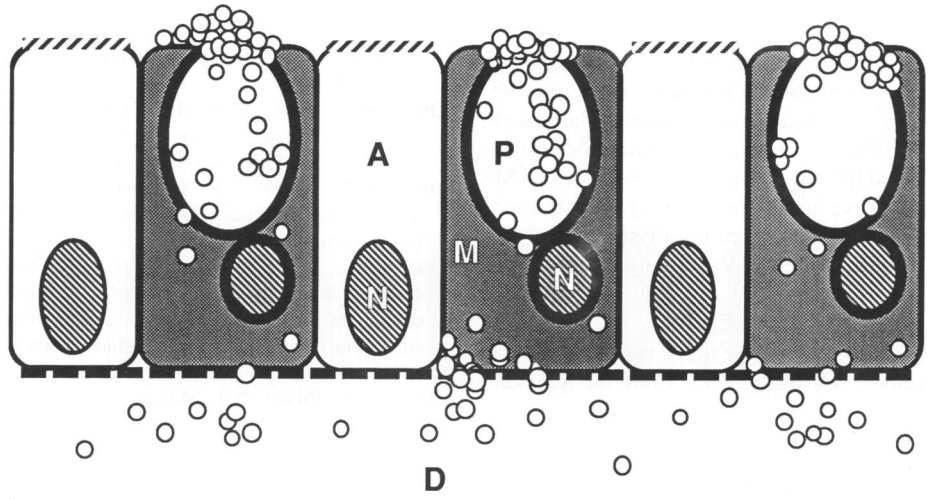

Fig. 2. Schematic diagram of relationship between fluorescent microspheres and follicle epithelial cells in rabbit Peyer's patch. M cells (M) alternate with absorptive cells (A). Particles are localized at the surface of M cells, within M cell pockets (P), and in the follicle dome (D). The MoAb label the pocket and perinuclear regions of M cells (represented by dense lines). N nucleus.

gates (Neutra *et al.*, 1987), and microorganisms (Wolf *et al.*, 1981; Owen *et al.*, 1986). The rapid rate of M cell transport suggests that the particles first adhered to M cells, since materials which bind to M cell membranes may be transported *in vivo* at least 50 times more efficiently than their non-adherent counterparts (Neutra *et al.*, 1987). The only exception to these findings is the interaction between FAE cells and *E. coli* strain RDEC-1, which attaches preferentially to M cell apical membranes, but is not phagocytosed (Inman & Cantey, 1983).

M cells have been found by ultrastructural analyses to be specialized sites for the recognition and phagocytosis of reovirus (Wolf *et al.*, 1981), *V. cholerae* (Owen *et al.*, 1986), *S. typhi* (Kohbata *et al.*, 1986), *M. paratuberculosis* (Momotami *et al.*, 1988), and *Cryptosporidium* (Marcial & Madara, 1986). Particulate microbial antigens localize to M cell apical surfaces, are engulfed by M cell pseudopods, and are shuttled in vesicles to underlying lymphocytes and antigen presenting cells. The relative deficiency of lysosomes in M cells, compared with adjacent enterocytes, has suggested that antigens taken up by M cells are not extensively catabolized during transit across the FAE (Owen, Apple & Bhalla, 1986), although the pathways of antigen handling by M cells have not been studied directly. The amount of antigen absorbed in the intestine, presumably across non-Peyer's patch epithelium, through non-receptor-mediated endocytosis has been estimated to range between 0·01% and 0·02% of the total antigen load (Gruskay & Cooke, 1955; Warshaw *et al.*, 1971). While soluble antigen is more effectively transported across Peyer's patch epithelium than across villus epithelium (Keljo & Hamilton, 1983), it has not been possible to assess quantitatively the magnitude of particulate uptake, and subsequent transport by M cells. We have now shown that Peyer's patch M cells, identified by immunolabelling with MoAb 5D9 and 1D9 (Pappo, 1989), take up an insoluble, easily traceable and quantifiable particle from the intestinal lumen, and transport it across the FAE into Peyer's patch domes. The relationship between the fluorescent particles and labelled M cell pocket regions is illustrated schematically in Fig. 2. We estimate this transport to occur at an average rate of 2 μm/min.

The finding of approximately 2000 fluorescent particles per mm length of Peyer's patch lymphoepithelial dome suggested a considerably greater efficiency of M cells for the 'non-specific' phagocytosis of polystyrene microspheres, compared with the uptake and transepithelial transport of other biological particles administered intraluminally at equivalent concentrations (Wolf *et al.*, 1981; Owen *et al.*, 1986). Also, particle uptake by rabbit Peyer's patches was at least one order of magnitude greater than that observed in murine Peyer's patches (unpublished). The findings reported herein suggest that each Peyer's patch lymphoepithelial dome harboured from $2–3 \times 10^5$ particles. Given that rabbit Peyer's patches contain 40–50 lymphoid nodules (Faulk *et al.*, 1970), it can be estimated that approximately 5% of the total intraluminal particles administered were carried into Peyer's patches.

Currently little is known about the dynamics of M cell antigen sampling and membrane recycling. The observations that the number of particles at the M cell apical region did not increase from 10 min to 90 min, despite the presence of free intraluminal particles, and that particles accumulated within subepithelial domes in a time-dependent fashion at the expense of the microspheres localized within the FAE surface, suggests that latex microspheres, and possibly other particulates, may be mobilized into Peyer's patches in a synchronous cycle lasting at least 90 min. The model described herein may allow understanding of the role of intestinal IgA, or IgA-antigen conjugates on M cell uptake, and may prove useful in studying quantitative relationships of antigen taken up (linked to polystyrene microparticles), and the magnitude of an IgA antibody response.

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