Kinetics of particle uptake in the domes of Peyer’s patches

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Beier, Rita, and Andreas Gebert. Kinetics of particle uptake in the domes of Peyer’s patches. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G130–G137, 1998.—Uptake of particulate antigenic matter, including microorganisms and vaccine-bearing microspheres, by the intestinal mucosa takes place in the domes of the gut-associated lymphoid tissues and is achieved by membranous (M) cells, which continuously transport particles from the lumen to the underlying tissue where some particle components initiate immune reactions. Using yeast as tracer, we investigated the kinetics of particle uptake in the Peyer’s patches of pigs. A suspension of baker’s yeast (Saccharomyces cerevisiae) was injected into the gut lumen of anesthetized minipigs; the position of yeast cells in the tissue was determined after 1, 2.5, 4, and 24 h using fluorescence light- and thin-section electron microscopy. After 1 h, 18.5% of all M cells had taken up or were in close contact with yeast cells. The intercellular space of the epithelium contained a maximum of 60.8% of all yeast cells found in the tissue after 2.5 h, but only 1.3% had been phagocytosed by macrophages. After 4 h most yeast cells (77.8%) were found beneath the basal lamina, and most of these (89%) were found in macrophages. No yeast cells were detected in the Peyer’s patch domes 24 h after application. The data show that transcytosis of yeast particles (3.4 ± 0.8 µm in diameter) by M cells takes <1 h. Without significant phagocytosis by intraepithelial macrophages, the particles migrate down to and across the basal lamina within 2.5–4 h, where they quickly get phagocytosed and transported out of the Peyer’s patch domes.

gut-associated lymphoid tissue; M cells; pig; infection; vaccination

The intestinal mucosa allows the passage of nutrients mostly in monomeric form but protects the organism from being invaded and damaged by toxins, microorganisms, and other pathogens (37). However, such matter is continuously sampled in the gut-associate lymphoid tissues to immunologically survey the gut content and to generate immune reactions against potentially harmful agents (20). The initial step in this process is antigen uptake by membranous (M) cells, e.g., in domes of the Peyer’s patches (28, 31). Although transepithelial transport by M cells has been thoroughly studied in the past few decades using soluble tracers, experimentally introduced bacteria, and inert particles (for review see Ref. 11), little is known about the kinetics of this uptake, the further pathway of the antigens into the domes, and the fate of particulate substances in the lymphoid tissue (see Ref. 18). Knowledge of such kinetics and interactions would not only be helpful for understanding and impeding microbial infections but also for the development of vaccines orally applied and taken up in the gut-associated lymphoid tissues. We therefore applied a large particulate tracer to the Peyer’s patches of pigs and followed its transport into the domes by electron microscopy (EM).

Baker’s yeast (Saccharomyces cerevisiae) was selected as tracer because its relatively large size matches that of microspheres experimentally used for the oral application of vaccines and drugs (5–7, 15). In contrast to these biodegradable vehicles, the yeast cells are relatively stable against solvent extraction effects (see Ref. 7), and, in contrast to particulate microbial antigens, relatively stable against digestion and lysis by macrophages (32, 34). Thus baker’s yeast can easily be identified in the tissue even after hours and days, as previously demonstrated for this uptake model (10). In addition, baker’s yeast is noninvasive and, in the normal situation, nonpathogenic and thus should not interfere with or predominate physiological uptake by gut-associated lymphoid tissues. Nevertheless, the clinical significance of baker’s yeast is underlined by reports on invasive infections in patients suffering from severe immunodeficiencies (3).

Thin-section EM was used to localize yeast cells in dome epithelium and in underlying lymphoid tissue because it allows identification of both the tracer and the different cell types of the host, e.g., M cells, enteroctyes, and macrophages. In contrast to the various techniques of light microscopy, EM furthermore allows the intra- or extracellular position of the tracer to be determined with regard to these cell types. The time kinetics of particle transport into the tissue were followed in vivo after application of yeast suspension to the gut lumen above Peyer’s patches at different intervals, i.e., 1, 2.5, 4, and 24 h. The normal ultrastructure of M cells in the porcine Peyer’s patches was determined, and the changes during particle uptake were documented.

MATERIALS AND METHODS

Animal experiments. Female minipigs (n = 10) of the Göttin gen breed, aged 3.5–8 mo, were kept under specified pathogen-free conditions. They were fed sterilized standard laboratory diet and had free access to water. The animals were laparotomized under intravenous thiobarbiturate anesthe sia (Trapanal, Byk Gulden, Konstanz, Germany) under sterile conditions. Isolated loops of about 25 mm in length were prepared in situ from the jejunum, each being supplied by one or two blood vessels and each including a discrete Peyer’s patch. Approximately 300 µl of a yeast suspension (see Yeast suspension) were injected into the lumen of each isolated loop of the jejunum in seven of the pigs; additional loops not injected with yeast were prepared in these animals and in two other pigs and served as controls. The gut was then returned to the abdominal cavity, and after 1, 2.5, and 4 h the
loops were removed and immediately opened, rinsed in Ringer solution for 10 s, and cut into small pieces with razor blades. In one of the pigs, no loops were prepared, but 10 ml of yeast suspension were injected into the duodenum. The pig was re-laparotomized after 24 h, and the Peyer’s patches were removed as described previously. All animals were killed by an intravenous injection of T-61 (containing embutramide, mebenzonium iodide, and tetracaine hydrochloride; Hoechst, Frankfurt, Germany). The animal experiments were approved by the local government of Lower Saxony, Germany (TS-No. 604–42502–94/719).

Yeast suspension. Lyophilized baker’s yeast (Saccharomyces cerevisiae; Dr. Oetker, Bielefeld, Germany) was suspended in PBS as described previously (10). To identify yeast by fluorescence microscopy, the rehydrated yeast was incubated with FITC (Sigma, Deisenhofen, Germany) at a concentration of 1 mg FITC per 100 mg rehydrated yeast for 1 h. Subsequently, free FITC was removed by at least four washes in PBS. Each isolated loop was injected with ~5 × 10⁵ yeast cells.

Electron microscopy. The tissue samples were fixed in a solution of 2% formaldehyde, 2.5% glutaraldehyde, and 2 mg/ml CaCl₂ in 0.1 M Na-cacodylate-HCl buffer, pH 7.3, at 4°C for 16 h. After being rinsed in cacodylate buffer for 30 min, the blocks were postfixed in 2% osmium tetroxide in cacodylate buffer, dehydrated in a graded series of ethanol dilutions, transferred to propylene oxide, and embedded in Epon (Serva, Heidelberg, Germany) according to standard protocols. Semithin sections, 1 µm in thickness, were stained with toluidine blue. To identify fluorescent yeast cells, semithin sections were examined with a fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with a filter set for FITC fluorescence. Ultrathin sections, 50–70 nm in thickness, were stained with uranin acetate and lead citrate and examined in a Zeiss EM-10 electron microscope at 80 kV.

Quantitative analyses. A total of 73 domes of yeast-injected animals and 28 domes of control animals were examined by light microscopy; 34 domes of yeast-injected animals and 6 domes of control animals were examined by EM. Each dome was investigated using two or more semithin sections and three or more ultrathin sections oriented perpendicular to the longitudinal axis of the dome and running across both its tip and its flank. For quantitation of cell types and ultrastructural characteristics a total of 1,849 dome epithelial cells were analyzed in the yeast-injected animals and 663 dome epithelial cells in the control animals. Descriptions and quantitations of M cell morphology were based on a total of 529 M cells. The number and position of yeast cells in the tissue were determined by fluorescence microscopy of 318 semithin sections taken from 73 domes. The position of 466 yeast cells in the epithelium and lymphoid tissue of the domes was analyzed by EM. The phrase “direct contact to M cell!” is used for yeast cells lying in the intercellular space of the dome epithelium at a distance of <5 µm from the basolateral membrane of an M cell. At the ultrastructural level, distances and diameters were measured on prints at a final magnification of 40,000:1 and are given as means ± SD.

RESULTS

The dome epithelia of yeast-injected and control pigs basically showed a similar cellular composition with enterocytes, M cells, intraepithelial lymphocytes, and macrophages (Figs. 1 and 2). The enterocytes were characterized by a dense brush border consisting of regular microvilli, 2.7 ± 0.5 µm (n = 153) in length and 141 ± 12 nm (n = 153) in diameter (Fig. 2). The M cells varied in surface morphology with some possessing irregular microvilli, 0.76 ± 0.4 µm (n = 97) in length and 187 ± 79 nm (n = 149) in diameter, and others possessing protrusions of the apical cytoplasm (Fig. 2). The apical cytoplasm of the M cells contained numerous round or oval vesicles, 82 ± 30 nm (n = 200) in diameter, and a few larger vacuoles, 200–750 nm in diameter. The M cells comprised 21.4% of all dome epithelial cells, the enterocytes 76.6%; goblet cells were rare (0.5%), and the remaining 1.5% of the dome epithelial cells could not be categorized. Intraepithelial lymphocytes and macrophages were identified by the typical size and shape and by the content of electron-dense lysosomes in the cytoplasm, respectively. In contrast to other species and locations investigated previously, clear distinctions between intraepithelial spaces and M cell pockets could not be made. Yeast cells, 3.4 ± 0.8 µm (n = 100) in diameter, were densely stained in semithin sections (Fig. 1) and possessed the typical cell wall and electron-dense cytoplasm in ultrathin sections (Figs. 2 and 3).

Uptake of yeast from the gut lumen was restricted to the dome epithelium (Fig. 1) and was almost exclusively performed by the M cells. In the yeast-injected animals, only two of the 1,400 dome epithelial enterocytes investigated (0.14%) contained yeast. In contrast, 18.5% of the M cells contained or were in direct contact with yeast cells after 1 h, decreasing to 12.3% after 2.5 h and to only 4% after 4 h. M cells that endocytosed yeast at the apical membrane typically formed cytoplasmic protrusions that almost engulfed the yeast cell and in some cases overreached the microvilli of neighboring enterocytes (Fig. 2B). Despite this generally observed pattern, there was considerable variation in the amount of yeast taken up between individual domes and individual animals; the number of yeast cells per section profile of a dome ranged from zero up to a maximum of 126. To facilitate the quantitative analysis of the transport kinetics, fluorescence microscopy of semithin sections was used to select section profiles that contained a minimum of four yeast cells. Of all 73 domes investigated, 46 domes contained yeast and were included in the quantitative analyses.

After 1, 2.5, and 4 h of incubation, yeast cells were present at different positions in the dome epithelium and beneath its basal lamina (Table 1). Yeast cells were found in contact with the apical membrane of M cells 1) lying in vacuoles in the cytoplasm of M cells, 2) in the intercellular space of the epithelium, and 3) in cytoplasmic vacuoles of intraepithelial macrophages; beneath the basal lamina, yeast cells were present 4) in the intercellular space and 5) in phagocytes of the lymphoid tissue. Only 9% of the 466 yeast cells investigated in total were found in the cytoplasm of M cells (Fig. 2), mostly present in the apical half (8.6%) and only rarely in the basal half (0.4%). In contrast, the intercellular
The space of the dome epithelium contained 42.7% of all yeast cells analyzed (Fig. 3), mostly located in the basal half (36.3%) and only infrequently in the apical half (6.4%). Only 1.3% of all yeast cells were found in intraepithelial macrophages of the dome epithelium, but macrophages of the lymphoid tissue beneath the basal lamina contained 15.7% of the yeast cells (Fig. 4). The remaining 31.3% of yeast cells lay beneath the basal lamina of the dome epithelium in the intercellular space of the lymphoid tissue. In the control pigs, no electron-dense structures resembling yeast, bacteria, or other particles were found in or adhering to the dome epithelium.

To determine the time kinetics of yeast transport into the domes, the distribution of yeast cells was calculated separately for each incubation time (Table 1). The percentage of yeast cells present in the cytoplasm of M cells decreased from 12.9% after 1 h to about 6% after 2.5 and 4 h. No yeast was found in M cells 24 h after yeast application. The percentage of yeast cells present in the intercellular space of the dome epithelium increased from 31% after 1 h to 60.8% after 2.5 h. Only minimal numbers of yeast cells were found in this compartment after 4 h, and no yeast cells were found after 24 h. The lymphoid tissue beneath the basal lamina of the dome epithelium contained 56.2% of the yeast cells after 1 h and 33% after 2.5 h. A maximum percentage of 77.8% of the yeast cells in this compartment was reached after 4 h. While after 1 h only 17% of all yeast cells lying beneath the basal lamina were
found in the cytoplasm of macrophages, the relative number of phagocytosed yeast cells increased to 31% after 2.5 h and to 89% after 4 h.

**DISCUSSION**

The transport of antigenic matter from the gut lumen into the lymphoid tissue has been studied in various species, at different locations of gut-associated lymphoid tissues, and with numerous particles serving as experimental tracers (review Ref. 11). It is unquestionable that the M cells of the dome epithelium play the key role in this transport (27, 31), but little is known about the significance of other cell types, such as macrophages, and about the kinetics of the transport of...

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**Fig. 2.** Thin-section electron microscopy of Peyer’s patch dome epithelium with enterocytes (E) and M cells (M). Whereas enterocytes possess relatively long, thin, regular microvilli, those of M cells are irregular in size and shape. A: yeast cells, when injected into gut lumen, came into contact with apical membrane of M cells and were phagocytosed. Magnification ×12,200. B: M cells containing yeast in cytoplasmic vacuoles often exhibited protrusions of apical cytoplasm, which probably resulted from rearrangement of the M cell cytoskeleton during endocytosis. The maximum percentage (18.5%) of M cells associated with yeast was found 1 h after application of yeast suspension. Magnification ×6,900.
particulate antigens. Data on these aspects of the induction of immune responses and immune tolerance would be advantageous for a better understanding of intestinal infections by microorganisms that invade the host via M cells (17, 33, 35, 39). At present several groups are endeavoring to develop oral vaccines using biodegradable microspheres and other particles as vehicles (4–7, 15). In the present study, we used a large-particulate, but stable tracer, baker’s yeast to follow its route into the lymphoid tissue and to determine its time kinetics.

The results show that the uptake of yeast is restricted to the domes of the Peyer’s patches and is not performed by the epithelium of ordinary villi and crypts. In the dome epithelium, the transepithelial transport of the yeast cells is almost restricted to the M cells. These findings are in accordance with previous studies on particle uptake in the intestine (7, 16) and further emphasize the significance of M cells. During endocytosis of yeast cells the M cells form pseudopodia-like protrusions of the apical cytoplasm, which engulf the yeast. This mechanism of particle uptake apparently differs from the pinocytosis of soluble antigens, such as horseradish peroxidase, lectins, ferritin, or cholera toxin (9, 29, 31), but resembles the phagocytosis of particles by typical phagocytes, such as macrophages (1, 8). Because similar cytoplasmic pseudopodia were described for the uptake of poly-(d-L-lactic-co-glycolic)-acid microspheres by rabbit M cells (7), it can be assumed that this represents a general mechanism for the phagocytosis of larger particles by M cells. The uptake of yeast by the Peyer’s patch M cells varied

Table 1. Distribution of yeast cells at different times after instillation

<table>
<thead>
<tr>
<th>Location of Yeast</th>
<th>1 h</th>
<th>2.5 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In the cytoplasm of M cells</td>
<td>12.9%</td>
<td>6.2%</td>
<td>6.7%</td>
</tr>
<tr>
<td>2. In the intercellular space of the epithelium</td>
<td>30.9%</td>
<td>60.8%</td>
<td>2.2%</td>
</tr>
<tr>
<td>3. In intraepithelial macrophages</td>
<td>0.0%</td>
<td>0.0%</td>
<td>13.3%</td>
</tr>
<tr>
<td>4. In the intercellular space beneath the basal membrane</td>
<td>46.4%</td>
<td>22.9%</td>
<td>8.9%</td>
</tr>
<tr>
<td>5. In phagocytes beneath the basal membrane</td>
<td>9.8%</td>
<td>10.1%</td>
<td>68.9%</td>
</tr>
</tbody>
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E, enterocyte; M, M cell; P, phagocytes; L, lymphocyte; b.m., basal membrane.
considerably between individual pigs and between individual domes of the same animal, as similarly observed for the uptake of latex microspheres in the rabbit (16). Because, in addition, <20% of all M cells were involved in yeast uptake, it can be assumed that the transport of particulates by M cells is variable and possibly regulated by still unknown factors. Further studies are needed to identify factors that induce or enhance the transport capacity of M cells, e.g., using cell culture models that have recently been introduced by Kernéis et al. (19).

Quantitative data on the distribution of a tracer after different incubation periods, as provided in the present study, allow the uptake kinetics of particulate antigens by the domes of the Peyer’s patches to be estimated. The maximum percentage of 18.5% of yeast cells associated with M cells found after 1 h of incubation suggests that the passage through the apical cytoplasm of the M cells is a relatively fast process taking about 1 h. This is in accordance with uptake experiments using latex particles and bacteria in a cell culture model (19) and biodegradable microspheres and latex particles in the gut-associated lymphoid tissue of other species (7, 16, 34). With the latter tracer and the rabbit model, the transport speed was determined to be 2 µm/min (34), and the total uptake capacity of a single rabbit Peyer’s patch dome was determined to be 10^5 latex particles in 45 min (16). In the pig most yeast cells (60.8%) had passed the M cells within 2.5 h and lay in the interstitial space of the dome epithelium. Because, in total, only 1.3% of the yeast cells were endocytosed by intraepithelial macrophages, it can be assumed that these play only a minor role in the transport of particulates. After 4 h, only 2.2% of the yeast cells were present in the intercellular space of the dome epithelium, but more than two-thirds of the cells had entered the subepithelial lymphoid tissue. Because baker’s yeast is immobile and probably only slightly affected by molecular diffusion, mechanisms remain to be established that allow the yeast cells, and likewise other particulates, to be transported down to and across the basal lamina. One such factor might be a solvent drag by fluid absorbed from the gut lumen by the enterocytes of the dome epithelium. The yeast cells, >3 µm in diameter, probably pass through pores of the basal lamina of the dome epithelium, which were previously described for the Peyer’s patches of rats, monkeys, and horses (22, 24, 25). The yeast cells reached the lymphoid tissue within about 4 h and were quickly phagocytosed by macrophages, so that only 11% of the yeast cells in this compartment lay in the intercellular space. The absence of yeast cells 24 h after application suggests that they had been completely phagocytosed and that the yeast-laden phagocytes had left the domes. It can be assumed that macrophages migrate to lymphatic vessels (23, 26, 30), leave the Peyer’s patches, and migrate to the draining lymph nodes, primarily to those of the mesentry. This assumption is supported by experiments with fluorescent microbeads that were found in macrophages of the draining lymph nodes 7 days after injection into the gut lumen (21, 38) and by studies with polystyrene and biodegradable microspheres, which suggest that a diameter of <5 µm is necessary for a particle to be transported to draining lymph nodes and other organs (5, 7, 13).

Fig. 4. Basal part of dome epithelium (top third) and lymphoid tissue underlying basal lamina (indicated by arrows). The majority of yeast cells lying in the subepithelial lymphoid tissue were present in cytoplasmic vacuoles of macrophages (asterisk). The maximum percentage of yeast cells found at this position (68.9%) was reached 4 h after yeast application. Note that on left-hand side basal membrane is discontinuous with lymphocyte (L) trafficking through pores. Magnification ×4,400.
The kinetics of yeast uptake show that it takes <1 h for a particulate antigen of the gut lumen to come into contact with cells of the lymphoid system and that the lymphoid cells of the Peyer’s patches have a few hours to initiate immune reactions. However, it is unclear at which site the antigen presentation takes place and which site the antigen presentation takes place and which cells are involved in this important step. It has been demonstrated that M cells of the rat possess molecules, e.g., major histocompatibility complex (MHC) class II and specific lysosomal enzymes, that enable M cells to degrade antigens in the lysosomal compartment and to present them to intraepithelial lymphocytes (2). Such antigen presentation within the dome epithelium has also been ascribed to the intraepithelial macrophages (2, 12, 14), but the present observations show that only extremely small amounts of antigens are phagocytosed within the epithelium. It seems to be more likely that phagocytosis, lysosomal degradation, and antigen presentation are performed by subepithelial phagocytes of the dome epithelium. Recent immunohistochemical findings suggest that a specialized subtype of dendritic cells is present in the subepithelial domes of murine Peyer’s patches and expresses 5- to 10-fold higher levels of MHC class II antigens than dendritic cells of other origin (18, 36). The methods used in the present study do not readily allow such dendritic cells to be identified, but it can be assumed that they are involved in the presentation of antigens previously transported and/or enzymatically degraded by macrophages and other cells. Nevertheless, it cannot be excluded that antigen presentation is mainly performed in the draining lymph nodes, because the antigenic matter is transported here via the lymphatics. These important questions on the site of antigen presentation should be addressed in further experiments, e.g., by resection of mesenteric lymph node and/or by collecting the lymph coming from Peyer’s patches.

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