Commentary

M Cells—Entryways of Opportunity
for Enteropathogens
By Robert L. Owen

From Cell Biology and Aging Section, VA Medical Center and the Department of Medicine,
University of California, San Francisco, California 94121

Peyer's patches have been thought of as areas of weakness in the intestinal wall since they were first noted in hog intestine used as casings for sausages (1). Butchers noted little bulging areas of decreased tensile strength in some regions of the casings, which were attributed to hogs swallowing too much air. These intestinal "glands" were mentioned by Hippocrates, and in 1677, Johann Conrad Peyer found that they corresponded to lymphoid nodules, interrupting the muscularis mucosae, which are now known as Peyer's patches. Clinically, Peyer's patches are also notable as areas of weakness in the mucosal barrier, especially as sites of intestinal penetration by Salmonella typhi, which is the etiologic agent of typhoid fever. In 1829, Louis described intestinal perforation as a primary complication of typhoid fever. He reported that perforation was usually single and located just above the cecum, in the center of an ulcer on the antimesenteric side of the ileum, which corresponds to the location of the major human Peyer's patch (2). In this issue of The Journal of Experimental Medicine, Jones et al. (3) provide new information regarding the pathogenic mechanism by which Salmonella spp. penetrate Peyer's patches and create micro-ulcerations, that allow intestinal flora to enter the epithelium and lamina propria of the ileum.

The pathology of typhoid infection has long held clues pointing toward a major pathway by which intestinal infectious agents can produce systemic illness. After infection with S. typhi, Peyer's patches in the terminal ileum typically develop hyperplasia after the first week, which can either resolve or progress to necrosis in the second week. Ulcers can lead to perforation and hemorrhage, usually in the third week. Although septicemia and constitutional symptoms produce considerable morbidity, perforation has been and remains a major cause of mortality in typhoid. A review of mortality in typhoid patients with intestinal perforation, between 1937 and 1983, identified 2,112 patients treated surgically with 28% mortality and 409 patients treated medically with 70% mortality (4).

Although typhoid has been largely eliminated in the industrialized world, it remains a major problem in third world countries. From 1975 to 1983 at the International Centre for Diarrhoeal Disease Research Hospital B in Dhaka, Bangladesh, 323 patients with typhoid were identified, of whom 15 (4.6%) developed intestinal perforation (4). A review of 57,864 cases of typhoid in the third world during the antibiotic era found 1,448 (2.5%) with perforation, which accounted for 25% of all typhoid deaths (5).

A histopathological study of 184 cases of intestinal perforation due to typhoid in Bandung, Indonesia, from 1972 to 1976, found 142 cases (77.2%) in males and 42 (22.8%) in females, mostly in young adults and uncommonly in children. Pathological change began as edema and hyperplasia of Peyer's patch lymphoid elements, with stretching of the overlying mucosa, surface erosions, and later necrosis of lymphoid tissue (6). Interestingly, isolation of S. typhi from the peritoneal cavity is rare in cases of perforation. Instead there is usually polymicrobial infection with aerobic gram-positive cocci, aerobic gram-negative bacilli, and also anaerobic bacteria, presumably from "normal" luminal flora leaking across the perforation (4).

Immunohistochemical examination of formalin-fixed ileal tissue from 13 patients with typhoid perforations, using rabbit polyclonal antisera to salmonella, failed to show any residual salmonella infection at the time of perforation (Mukawi, T. J., D. F. Keren, and R. L. Owen, unpublished data). The pathophysiological basis for this remarkable association of perforation with Peyer's patches in the terminal ileum has long remained a source of puzzlement and speculation.

Twenty years ago, Peyer's patches, tonsils, and the appendix were considered vestigial organs without known function, important only as possible sites of suppuration. New information regarding mechanisms of bacterial attachment and entry into host cells, and advances in understanding the structure and function of mucosal components of the intestinal immune system, have explained how salmonella attach to enterocytes and why uptake takes place in Peyer's patches (7). Kumagai (8) had noted in 1922 that Mycobacterium tuberculosis fed to rabbits were taken up by lymphoid follicles in Peyer's patches and in appendix, and the same process, was seen with powdered red blood cells, suggesting that it was a general phenomenon and not a specific mechanism of bacterial invasion. Peyer's patches in human ileum, the avian bursa of Fabricius, and the rabbit appendix were subsequently found to contain epithelial cells over lymphoid follicles, which are specially adapted for uptake of microbes and other particles from the intestinal lumen (9, 10). These cells, termed M cells, initially because of "microfolds" over their luminal surfaces in the human terminal ileum, are now thought of as "membranous", because lymphoid cells invaginating their basolateral surfaces compress them into attenuated bands, which like membranes, separate the intestinal lumen from tissue compartments, yet let particles and molecules cross in a controlled manner. M cells rest on the basal lamina and adhere to adja-
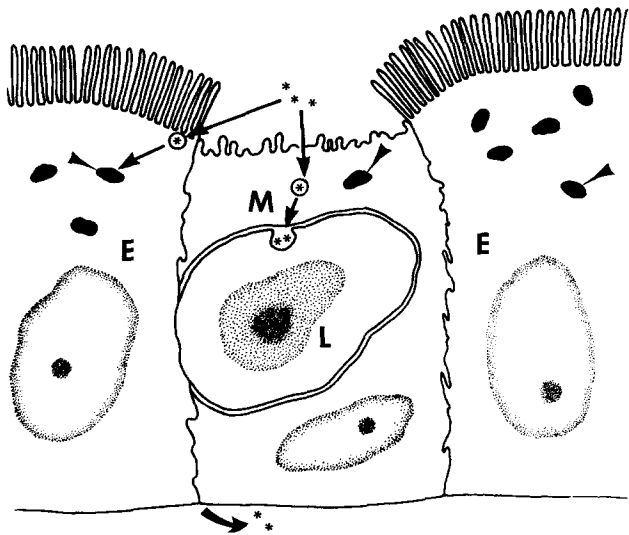
Figure 1. Uptake and transport of luminal microbes and other particles by M cells (*M*). The M cell has few lysosomes (*arrowheads*) compared with adjacent enterocytes (*E*). When particles (*asterisks*) are taken up by enterocytes, they are diverted into lysosomes and digested. Particles transported through M cells in vesicles are released into the extracellular space where they are taken up by lymphoid cells (*L*) invaginating basolateral surfaces of M cells or pass through the basal lamina (*curved arrow*). (Figure courtesy of Wiley-Liss, a division of John Wiley & Sons, Inc. [11]).

cent absorptive columnar cells (enterocytes). In contrast to enterocytes, M cells have a more open apical surface with less glycocalyx and more widely spaced microvilli. These features of M cells allow the approach of microorganisms and other intestinal luminal particles, which are kept at bay by the closely packed microvilli and thick glycocalyx of enterocytes. M cells phagocytose and endocytose microorganisms that reach their apical surfaces, and have reduced lysosomal degradation and a vesicular transport mechanism that delivers particles from the intestinal lumen to lymphoid cells nestled in invaginations in the basolateral membranes of M cells (11) (Fig. 1). After uptake and transport of microorganisms by M cells, it is thought that digestion and processing of such organisms to antigenic components begins in lymphoid follicle domes, and initiates mucosal immunologic responses. There is evidence that for some molecules, antigen processing may also take place in M cells (12), which function as the antigen-receiving surface for mucosal lymphoid tissue in lung, the eye, and the nasal epithelium, as well as in the intestine (for a review see reference 7).

As this complex system has evolved to protect animals from enteropathogens, parallel evolution of microorganisms has produced species, including *S. typhi*, *Yersinia enterocolitica*, and reovirus, capable of selective attachment to M cells and armed with mechanisms for evading host defenses (for reviews see references 7 and 13). *S. typhi* were found by Kohbata et al. (14) to adhere to M cell surfaces in ligated ileal loops of mice, with ballooning and destruction of M cells within 30 min. The relative contributions of microbial attachment mechanisms and of host antigen sampling and uptake mechanisms have remained uncertain for pathogens such as salmonella, because of the known capability of M cells for taking up noninvasive bacteria such as *Vibrio cholerae* (15).

In the current issue, Jones et al. (3) compared the fate of *Salmonella typhimurium* (which causes diarrhea in humans and a typhoid fever–like syndrome in mice) with a related genetically altered noninvasive strain, and found that the noninvasive strain was not taken up by M cells, demonstrating the importance of microbial factors in invasion of Peyer's patch follicles. The invasive strain attached preferentially to M cells and induced ruffling of M cell apical membranes, facilitating uptake of the attached *S. typhimurium*. The authors also found that the invasive strain, which was unable to pass the thicket of microvilli on the apical surface of enterocytes, was able to penetrate and destroy these enterocytes via their lateral membranes after destroying adjacent M cells. Resulting ulcerations over Peyer's patch lymphoid nodules provided an entryway for other enteric organisms. This work takes a long step in showing how salmonella produce the ulceration of Peyer's patches, initiating the pathologic cascade which can eventuate in intestinal perforation in *S. typhi* infection. Further investigation of factors governing salmonella attachment to M cells and salmonella induction of uptake by M cells will be important in devising better vaccines against *S. typhi*, and also in engineering salmonella mutants capable of carrying bacterial and viral immunogens of interest, including HIV surface antigens (16). Recombinant gene expression technology is now being used to introduce foreign bacterial, parasitic, and viral antigens into attenuated strains of *S. typhimurium*, which can colonize gut-associated lymphoid tissues, with the aim of initiating immune responses, protective for mucosal surfaces that are the entry pathways for most pathogens (17, 18).

References

1. Ten Cate, C.L. 1969. Casings with "patches of Peyer". ΠΕΡΙ ΑΔΕΝΩΝ: From sausages to Hippokrates. *Janus.* 56:22.
2. Louis, P.Ch.A. 1829 (in French). Perforations of the small intestine. *In* Anatomical, Pathological and Therapeutic Researches upon the Disease Known under the Name of Gastroenterite, Putrid, Adynamic, Ataxic, or Typhoid Fever, etc., compared with the most common acute diseases. [1836 translation, H.I. Bowditch. Vol. 2, Hilliard, Gray and Co., Boston 373–88].
3. Jones, B.D., N. Ghori, and S. Falkow. 1994. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized M cells of the Peyer's patches. *J. Exp. Med.* 180:15.
4. Bitar, R., and J. Tarpley. 1985. Intestinal perforation in typhoid fever: a historical and state-of-the-art review. *Rev. Infect. Dis.* 7:257.
5. Butler, T., J. Knight, S.K. Nath, P. Speelman, S.K. Roy, and M.A.K. Azad. 1985. Typhoid fever complicated by intestinal perforation: a persisting fatal disease requiring surgical management. *Rev. Infect. Dis.* 7:244.
6. Mukawi, T.J. 1978. Histopathological study of typhoid perforation of the small intestines. *Southeast Asian J. Trop. Med. Public Health.* 9:252.
7. Kato, T., and R.L. Owen. 1994. Structure and function of intestinal mucosal epithelium. *In* Handbook of Mucosal Immunology. R. Ogra, J. Mestecky, M. Lamm, W. Strober, J. McGhee, and J. Bienenstock, editors. Academic Press, Inc., Orlando, FL. 11–26.
8. Kumagai, K. 1922. Über den resorptionsvergang der corpusculären bestandteile im darm. *Kekkaku-Zassi (Japan).* 4:429 (Abstracted in *Berichte über die gesamte Physiologie und experimentelle Pharmakologie.* 1923;17:414.
9. Owen, R.L., and A.L. Jones. 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology.* 66:189.
10. Bockman, D.E., and M.D. Cooper. 1973. Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix and Peyer's patches. An electron microscopic study. *Am. J. Anat.* 136:455.
11. Owen, R.L., R.T. Apple, and D.K. Bhalla. 1986. Morphometric and cytochemical analysis of lysosomes in rat Peyer's patch follicle epithelium: their reduction in volume fraction and acid phosphatase content in M cells compared to adjacent enterocytes. *Anat. Rec.* 216:521.
12. Allan, C.H., D.L. Mendrick, and J.S. Trier. 1993. Rat intestinal M cells contain acidic endosomal-lysosomal compartments and express class II major histocompatibility complex determinants. *Gastroenterology.* 104:698.
13. Fujimura, Y., T. Kihara, and H. Mine. 1992. Membranous cells as a portal of *Yersinia pseudotuberculosis* entry into rabbit ileum. *J. Clin. Electron Microscopy.* 25:35.
14. Kohbata, S., H. Yokoyama, and E. Yabuuchi. 1986. Cytopathogenic effect of *Salmonella typhi* GIFU 10007 on M cells of murine ileal Peyer's patches in ligated ileal loops: an ultrastructural study. *Microbiol. Immunol.* 30:1225.
15. Owen, R.L., N.F. Pierce, R.T. Apple, and W.C. Cray, Jr. 1986. M cell transport of *Vibrio cholerae* from the intestinal lumen into Peyer's patches: a mechanism for antigen sampling and for microbial transepithelial migration. *J. Infect. Dis.* 153:1108.
16. Gilligan, C.A., and A. Li Wan Po. 1991. Oral vaccines: design and delivery. *Int. J. Pharm. (Amst.)* 75:1.
17. Cárdenas, L., and J.D. Clements. 1992. Oral immunization using live attenuated *Salmonella* spp. as carriers of foreign antigens. *Clin. Microbiol. Rev.* 5:328.
18. Schödel, F. 1992. Prospects for oral vaccination using recombinant bacteria expressing viral epitopes. *Adv. Virus Res.* 41:409.