Kerfoot and A. Sih, Eds. (Univ. Press of New England, Hannover, NH, 1987), pp. 17–30.
26. M. P. Hassell and R. M. May, *J. Anim. Ecol.* **43**, 567 (1974).
27. M. A. Hixon and J. P. Beets, *Ecol. Monogr.* **63**, 77 (1993).
28. As detailed elsewhere (*10*), we placed two Hi-8 video camera units underwater such that each viewed an entire experimental reef from the side, recording 15 s out of every 90 s from dawn to dusk. On 13 dates during the 1996 experiment, we conducted paired video samples of uncaged reefs with low (mean ± SEM = 5.4 ± 0.4 fish per reef) versus high (20.4 ± 1.7 fish per reef) experimental densities of newly settled *Chromis*.
29. We do not believe that this discrepancy could be due to nonbehavioral mechanisms, such as variable resident predator density. The densities of resident piscivores were unmanipulated in the two treatments where this suite of predators was present, so there was indeed natural variation among reefs in resident predator density. However, there was no significant difference in resident predator density between the treatment where all predators were present (mean ± SEM = 9.8 ± 1.3 fish per reef) and the treatment where only resident predators were present (11.0 ± 2.7 fish per reef, Mann-Whitney *U*-test, $P = 0.88$, $n = 8$ reefs each). Also, there was no correlation among reefs within either treatment between the density of resident piscivores and per capita settler mortality (correlation coefficient, $r_s = -0.21$, $P = 0.61$ and $r = -0.42$, $P = 0.31$, respectively).
30. A. C. Hurley and P. H. Hartline, *Anim. Behav.* **22**, 430 (1974); E. S. Hobson and J. R. Chess, *Fish. Bull.* **76**, 133 (1978); M. Hixon and M. Carr, personal observation.
31. W. P. Davis and R. S. Birdsong, *Helgo. Wiss. Meeresunters.* **24**, 292 (1973); P. G. Sackley and L. S. Kaufman, *Copeia* **1996**, 726 (1996). Midwater is safe for small fishes because resident predators tend to stay in cover, being prey of very large transient predators, especially great barracuda (*Sphyraena barracuda*) at our study site. We often observed barracuda attacking and capturing resident piscivores, and barracuda tooth scars were common on these fish. Barracuda ignored new settlers.
32. P. J. Doherty and T. Fowler, *Science* **263**, 935 (1994).
33. W. J. Richards and J. A. Bohnsack, in *Large Marine Ecosystems: Patterns, Processes, and Yields*, K. Sherman, L. M. Alexander, B. D. Gold, Eds. (American Association for the Advancement of Science, Washington, DC, 1990), pp. 44–53; G. R. Russ, in *The Ecology of Fishes on Coral Reefs*, P. F. Sale, Ed. (Academic Press, San Diego, CA, 1991), pp. 601–635; S. Jennings and J. M. Lock, in *Reef Fisheries*, N. V. C. Polunin and C. M. Roberts, Eds. (Chapman & Hall, London, UK, 1996), pp. 193–218.
34. S. L. Pimm and J. B. Hyman, *Can. J. Fish. Aquat. Sci.* **44**, 84 (1987).
35. For backbreaking work, we thank G. Almany, T. Anderson, J. Beets, R. Buckley, M. Buckley, B. Byrne, D. Canestro, G. Forrester, R. Gomez, A. Kaltenberg, A. Menge, G. Savage, S. Thompson, C. Tinus, E. Tynes, K. Vicknair, and M. Webster, and for general support, the staff of the Caribbean Marine Research Center, especially C. Cooper, G. Dennis, W. Head, W. Keith-Hardy, R. Wicklund, and T. Wolcott. Thanks to G. Almany, T. Anderson, K. Bailey, P. Bailey, J. Beets, C. Briggs, B. Byrne, J. Lubchenco, D. Reed, W. Pearcy, S. Sogard, and M. Webster for constructive reviews. Supported by NSF (OCE-92-17163 and OCE-96-17483) and National Oceanic and Atmospheric Administration's National Undersea Research Program (CMRC-92-46, 93-12, 94-15, 95-3042, and 97-3109).

17 April 1997; accepted 1 July 1997

Conversion by Peyer's Patch Lymphocytes of Human Enterocytes into M Cells that Transport Bacteria

Sophie Kernéis,* Anna Bogdanova,† Jean-Pierre Kraehenbuhl, Eric Pringault*‡

The epithelium that lines the gut is impermeable to macromolecules and microorganisms, except in Peyer's patches (PPs), where the lymphoid follicle-associated epithelium (FAE) contains M cells that transport antigens and microorganisms. A cultured system that reproduces the main characteristics of FAE and M cells was established by cultivation of PP lymphocytes with the differentiated human intestinal cell line Caco-2. Lymphocytes settled into the epithelial monolayer, inducing reorganization of the brush border and a temperature-dependent transport of particles and *Vibrio cholerae*. This model system could prove useful for intestinal physiology, vaccine research, and drug delivery studies.

Most microorganisms must cross epithelial barriers to exert their physiopathological effects and to interact with mucosa-associated lymphoid tissue (MALT). In the intestine, PPs are the major sites of antigen and microorganism sampling, which leads to immune responses or tolerance (*1*, *2*). MALT is separated form the lumen by the FAE, which contains M cells. These epithelial cells transport foreign material to MALT and display large intraepithelial pockets filled with B and CD4 T lymphocytes, macrophages, and dendritic cells. Many pathogenic microorganisms exploit M cells to cross the digestive epithelial barrier (*3*). Thus, passage of antigens and microorganisms through M cells is an essential step for the development of mucosal immune responses and the pathology of many infectious diseases. Because M cells are a minor population in the FAE, they are difficult to characterize biochemically; hence, little is known about their cell biology. The ultrastructure and the transport capacity of M cells have been documented by morphological analysis and immunohistochemistry. However, the study of molecular mechanisms of microorganism–M cell interactions and signal transduction pathways that control translocation and cytoskeletal reorganization has been hampered by the lack of in vitro M cell models. Here we report the establishment of an in vitro model in which specific properties of FAE and M cells can be analyzed by biochemical and quantitative methods.

The analysis of MALT and FAE in immunodeficient mice (*4*) suggests that FAE and M cell formation could be regulated by the presence of immune cells. A subclone of the human, differentiated, absorptive, enterocyte cell line Caco-2 clone 1 (*5*, *6*) was cultured with freshly isolated murine PP lymphocytes. We seeded Caco-2 cells by adding 3×10^5 cells on the lower face of 6.5-mm filters (3-μm pore Transwell filters, COSTAR, Cambridge, MA) and culturing them overnight. The filters were then transferred in the Transwell device with the epithelial cells facing the lower chamber of the cluster plates, as adapted from Kaoutzani *et al.* (*7*). Epithelial cells were cultured until they were fully differentiated (14 days). Lymphocytes were isolated from PPs of BALB/c mice (*8*). Dissociated cells were analyzed by FACScan (fluorescent analyzer cell sorter) flow cytometry (Becton-Dickinson). Sixty percent of the cells were B cells [detected with a monoclonal antibody (mAb) to mouse B220 (CD45)], and the remaining 40% of cells were CD3 T cells (detected with a mAb to mouse CD3). This ratio corresponds to that described for PP follicle cells (*9*). Macrophages and dendritic cells, representing about 0.4% of follicle cells in such preparations, were not detected. The lymphocytes survived in culture for up to 7 days. Lymphoid cells (10^6) were added in the upper chamber facing the basolateral side of the Caco-2 cells. The cultures were maintained for 1 to 7 days.

Lymphoid cells migrated through the pores of the filter and settled into the epithelial monolayer without altering the polarity of Caco-2 cells, as reflected by the maintenance of cell polarity markers (Fig. 1, A through C) and of transepithelial electric resistance of the monolayers (about 300 ohm · cm^2) during the 7 days of culture. Within 2 days, the lymphocytes accumulated in intraepithelial pockets, as already de-

Swiss Institute for Experimental Cancer Research, and Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges-Lausanne, Switzerland.

*Present address: Department of Bacteriology and Mycology, Pasteur Institute, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.
†Present address: INSERM U445, 22 rue Méchain, 75014 Paris, France.
‡To whom correspondence should be addressed. E-mail: epringau@pasteur.fr

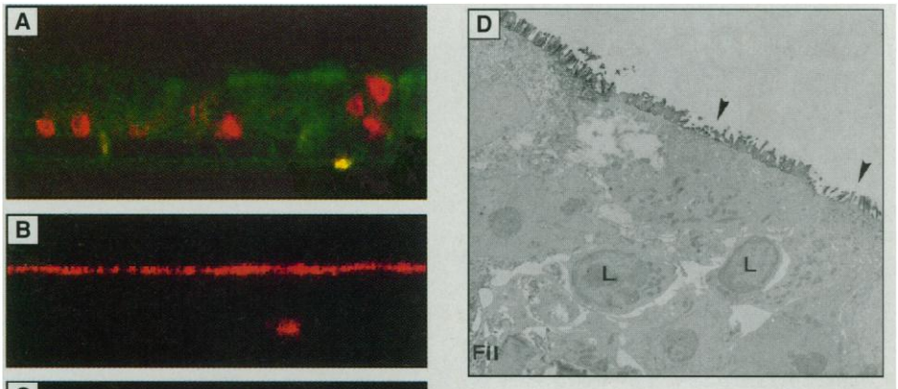

Fig. 1. Culture of PP lymphocytes with Caco-2 monolayers. (**A**) Immunocytochemical detection of B lymphocytes [Cy3-conjugated goat antibody to mouse IgG (10 μg/ml), Jackson Dianova] in a Caco-2 monolayer stained with FITC-conjugated UEA1 lectin (10 μg/ml, Sigma), analyzed by a Zeiss confocal microscope (XZ section). (**B**) Immunocytochemical detection of dipeptidyl-peptidase IV (DPP IV) (mAb to human DPP IV) (*29*), analyzed by confocal microscopy (XZ section). (**C**) Immunocytochemical detection of Na^+,K^+-ATPase (mAb to human Na^+,K^+-ATPase), analyzed by confocal microscopy (XZ section). (**D**) Transmission electron microscopy view of an ultrathin section of the coculture. Arrows indicate the apex of epithelial cells in contact with lymphocytes. L, lymphocytes; Fil, filter. Monolayers were fixed in a 2.5% glutaradehyde and processed for transmission electron microscopy, and ultrathin sections were examined with a JEOL JSM-35 electron microscope.

scribed in M cells in vivo. The presence of lymphocytes was correlated to the disorganization of apical microvilli of Caco-2 clone 1 cells (Fig. 1D), which normally develop well-organized brush borders (*5*, *6*). We analyzed the expression of two proteins that reflect the brush-border organization: villin, an actin-associated protein playing a key role in brush-border cytoskeleton assembly (*6*, *10*); and sucrase-isomaltase (SI), a brush-border hydrolase produced in differentiated small intestinal enterocytes (*11*, *12*). After 2 days of coculture, we observed by immunocytochemistry that villin and SI disappeared from the apical domain of Caco-2 cells (Fig. 2, A, B, E, and F). We performed quantitative studies of these proteins by immunolabeling with ^{125}I–protein A after cell permeabilization or by protein immunoblot analysis, both of which methods showed that the intracellular steady-state concentration of villin (Fig. 2, C and D) was not modified, whereas that of SI was decreased (Fig. 2, G and H). Thus, the two proteins behaved differently: Villin was redistributed from the brush border to the cytoplasm as observed in vivo (*13*), whereas the SI steady-state con-

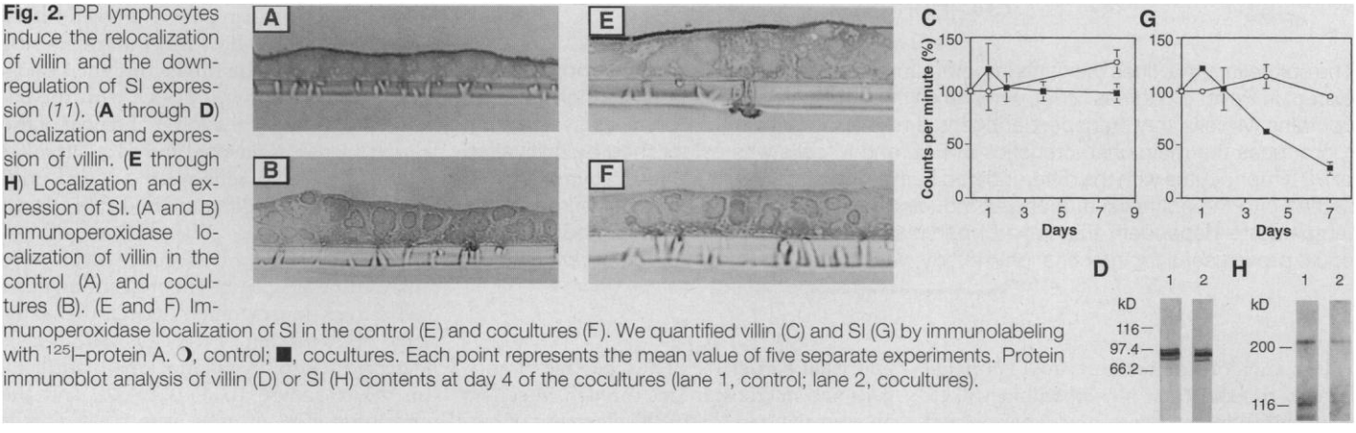

Fig. 2. PP lymphocytes induce the relocalization of villin and the down-regulation of SI expression (*11*). (**A** through **D**) Localization and expression of villin. (**E** through **H**) Localization and expression of SI. (A and B) Immunoperoxidase localization of villin in the control (A) and cocultures (B). (E and F) Immunoperoxidase localization of SI in the control (E) and cocultures (F). We quantified villin (C) and SI (G) by immunolabeling with ^{125}I–protein A. ❍, control; ■, cocultures. Each point represents the mean value of five separate experiments. Protein immunoblot analysis of villin (D) or SI (H) contents at day 4 of the cocultures (lane 1, control; lane 2, cocultures).

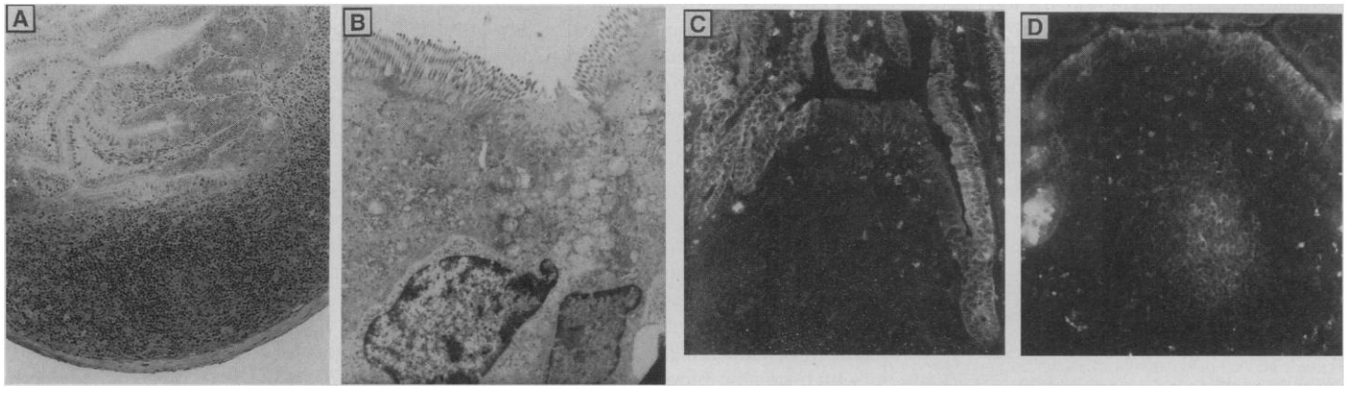

Fig. 3. Conversion of the villus epithelium into FAE after injection of PP lymphocytes in the duodenal mucosa of recipient mice. (**A**) Hematoxylin-eosin coloration of a semithin section of an induced PP-like structure 9 days after lymphocyte injection. (**B**) Transmission electron microscopy view of a thin section of an induced PP-like structure, showing a typical M cell with adjacent enterocytes. (**C** and **D**) Eight-micrometer frozen sections of induced PP-like structures. (C) Immunocytochemical detection of polymeric Ig receptor expression. (D) Fluorescence detection of bound FITC-conjugated UEA1 lectin.

centration was down-regulated. Digestive functions (nutritional enzymes and membrane transporters) in all epithelial cells of the FAE are reduced (*14–17*). That these changes were not restricted to the cells that were obviously in contact with lymphocytes, but probably affected all the cells in the monolayers, suggested that both soluble factors and cell contact were required for the conversion of absorptive enterocytes into FAE cells.

The ability of PP lymphocytes to convert enterocytes into M cells was also assessed in vivo. Lymphocytes freshly isolated from PPs were injected into the duodenal mucosa of recipient mice at sites lacking organized lymphoid tissues (*18*). When these sites were examined 9 days later, PP-like structures had developed (Fig. 3A), which consisted of well-organized lymphoid follicles with typical germinal centers and parafollicular regions. Antibody labeling with lymphocyte markers showed that the distribution of immune cells reflected that of endogenous PPs with a majority of CD4 T cells in the dome region, an enrichment of CD8 T cells in the parafollicular region, an accumulation of immunoglobulin M (IgM) B cells in the follicles, and IgA B cells restricted to the germinal centers (*19*). Some cells in the epithelium of these PP-like structures had a disorganized brush border and an intraepithelial pocket filled with lymphoid cells (Fig. 3B) that is characteristic of M cells. This epithelium expressed other features typical of the FAE, such as the lack of polymeric Ig receptors on all epithelial cells (Fig. 3C) and binding sites for the lectin Ulex Europaeus Agglutinin 1 (UEA1) on the membrane of a subpopulation of epithelial cells (Fig. 3D). The de novo induction of PP-like structures was consistently observed after the injection of PP lymphocytes (eight out of nine mice) but not after the injection of thymocytes (zero out of three mice). Splenocytes occasionally (one out of three mice) triggered the formation of small follicles, although these never reached the size of the structures induced by PP lymphocytes. These observations support the hypothesis that PP lymphocytes play a key role in induction of FAE and M cells. The normal distribution of PPs in the mouse intestine might be dictated by the presence of specialized endothelial cells, that is, the high endothelial venules that control the extravasation of immune cells that organized into MALT. In our experiments, the extravasation event was bypassed by direct injection of cells into the mucosa. FAE and M cell differentiation could be induced from any intestinal crypt, provided that appropriate lymphocytes or their secreted factors can gain access to the epithelium. In these experiments, we could not determine whether the formation of M cells was induced by events occurring in the local crypts or by conversion of differentiated absorptive enterocytes.

We next analyzed another distinct feature of M cells, the transport of inert particles (*20*), in the cocultures of Caco-2 with PP lymphocytes. Fluorescein isothiocyanate (FITC)–conjugated latex beads were introduced in the chamber facing the apical surface of epithelial cells (*21*). Fluorescence recovered from the opposite compartment was quantified by FACScan flow cytometry (Fig. 4). Transcytotic activity was monitored for 60 min at 4°C and after a rapid temperature shift to 37°C. In control monolayers, no latex beads were transported through the monolayers at either temperature (Fig. 4A). In the presence of lymphocytes, Caco-2 cells acquired transcytotic activity only at 37°C, resulting in a rapid accumulation of beads in the basolateral compartment after a lag period of 10 min. This temperature-dependent transcytotic activity reached its maximum after 3 or 4 days of coculture. No transport of latex beads occurred across Caco-2 cells that were unable to assemble a brush border (*19*), because of the suppression of villin production by villin antisense RNA (6). The absence of brush border was not sufficient to render the Caco-2 cells competent to transcytose microparticles. Thus, lymphocytes activated two distinct cellular effectors, one

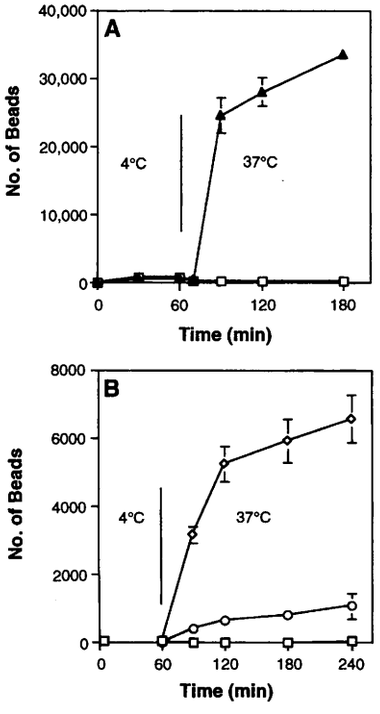

Fig. 4. Lymphocytes induce a temperature-dependent vectorial translocation of latex beads through Caco-2 monolayers. (**A**) Kinetics and temperature dependence of fluorescent latex bead translocation in the basolateral chamber induced by PP lymphocytes. □, control; ▲, cocultures. (**B**) Same experiment as in (A) but with human B (Raji) and T (Jurkat) lymphoid cell lines instead of mouse PP lymphocytes. □, control; ❍, Jurkat cells; ◊, Raji cells.

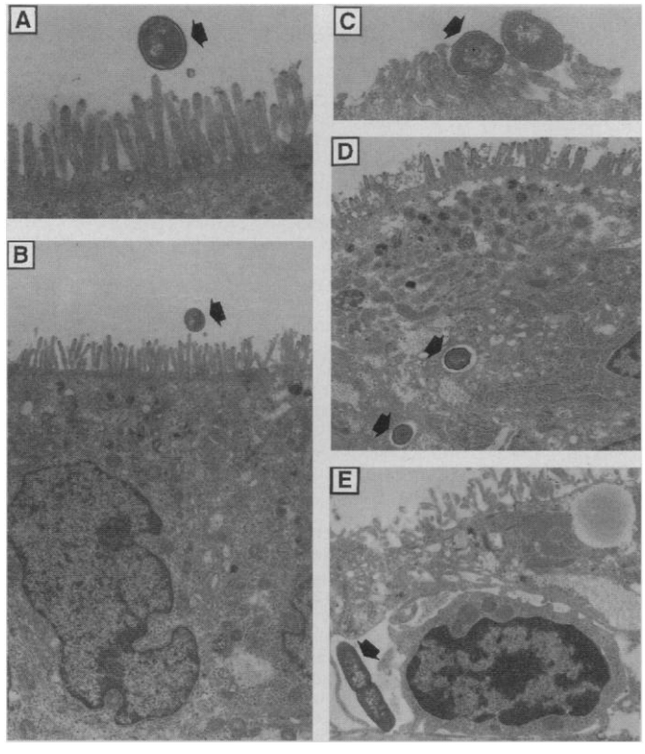

Fig. 5. *Vibrio cholerae* binding, internalization, and translocation. *Vibrio cholerae* was added for 60 min at 37°C into the lower chamber facing the apical surface of Caco-2 cells in the presence or absence of PP lymphocytes. Cells were then processed for transmission electron microscopy. (**A** and **B**) Control. (**C** through **E**) Cocultures. Arrows indicate bacteria.

that mediated brush border disassembly and a second that triggered transcytosis. In an homologous system, a mouse intestinal crypt cell line ICcl-2 (*22*), cultured with mouse PP lymphocytes, exhibited a similar transcytotic activity of inert particles (*19*). Because murine PP lymphocytes induced phenotypic conversion of both mouse and human enterocytes, the inductive factors and their corresponding receptors must be conserved between the two species.

Both B and T cells could cross the filters and settle into the monolayers. The respective contribution of B or T cells in Caco-2 cell conversion was analyzed with two human lymphoid cell lines (Fig. 4B). Transcytotic activity of inert particles was significantly triggered by Raji cells exhibiting B cell markers. In contrast, low transcytotic activity was induced by Jurkat cells with T cell markers. This difference suggests that cells bearing B cell markers are major inductive partners. Previous experimental data support a major role for B cells: adoptive transfer of B cells rather than T cells was more efficient in reconstitution of MALT structures and M cells in mice with severe combined immunodeficiency disease (*23*). In control experiments, isolated mouse thymocytes, human neutrophils, or HeLa cells were unable to induce transcytosis in the Caco-2 monolayers (*24*).

We analyzed the adhesion, internalization, and translocation of *Vibrio cholerae* O:1 (Ogawa), a noninvasive pathogen transported exclusively by M cells (*25*), after adding the bacteria to the apical side of Caco-2 cell in the presence or absence of lymphocytes (*26*). *Vibrio cholerae* did not alter the integrity of the epithelial cells, as reflected by the unchanged transepithelial electrical resistance after 2 hours of infection (292 ± 6 ohm · cm^2 in controls and 313 ± 8 ohm · cm^2 in cocultures). Infection was first performed at 4°C, and the temperature was then rapidly shifted at 37°C. Sampling of the basolateral medium after 1 hour of incubation at 4°C did not reveal the presence of bacteria in either the controls ($n = 3$) or the cocultures ($n = 3$). One hour after the shift at 37°C, internalization of *V. cholerae* increased by eight times in the presence of lymphocytes [$9.5 \pm 1.9 \times 10^3$ colony-forming units (CFU) cm^2 in controls ($n = 5$) and $78 \pm 35 \times 10^3$ CFU/cm^2 in cocultures ($n = 5$)]. Translocation of bacteria through the Caco-2 monolayers was stimulated about 100 times in the presence of lymphocytes [$1.92 \pm 0.72 \times 10^5$ CFU/cm^2 in controls ($n = 3$) and $204 \pm 39 \times 10^5$ in cocultures ($n = 3$)].

Because the number of intracellular bacteria represented a small fraction of the inoculum (0.01%) compared to the high percentage of translocated bacteria (20%), one has to assume that once internalized, the bacteria were rapidly transported and released into the basolateral compartment. The number of *V. cholerae* that attached to the apical surface of Caco-2 cells, analyzed by confocal microscopy, was not different in the presence or absence of PP lymphocytes (*19*), as described for *V. cholerae* in situ (*25*). Nevertheless, electron microscopy revealed a close contact of *V. cholerae* with the apical membrane of Caco-2 cells in the presence but not in the absence of PP lymphocytes (Fig. 5A). *Vibrio cholerae* were never detected intracellularly in control Caco-2 cells (Fig. 5B), whereas in converted monolayers, the intracellular bacteria were found in large vacuoles (Fig. 5D), which supports the hypothesis that vesicular transepithelial transport has occurred. Occasionally, *V. cholerae* were found in the intraepithelial pockets in the vicinity of immune cells (Fig. 5E).

In conclusion, major features of M cells, that is, vectorial translocation of inert particles and bacteria, have been reproduced in vitro by coculture of PP lymphocytes with enterocytes. By using this system, we observed a decrease of the steady-state concentration of SI and a cytosolic redistribution of villin, and we quantified the rate of translocation of a microorganism and inert particles. It should now be possible to identify the signals produced by lymphocytes responsible for M cell formation, to characterize the mechanisms mediating cytoskeletal reorganization and transcytosis, and to analyze the cellular machinery mediating microorganism translocation through M cells. This should facilitate the design of oral vaccines and efficient mucosal drug delivery systems.

REFERENCES AND NOTES

1. J. P. Kraehenbuhl and M. R. Neutra, in *Handbook of Mucosal Immunology*, P. L. Ogra *et al.*, Eds. (Academic Press, New York, 1994), pp. 403–410.
2. M. R. Neutra, E. Pringault, J. P. Kraehenbuhl, *Annu. Rev. Immunol.* **14**, 275 (1996).
3. A. Siebers and B. B. Finlay, *Trends Microbiol.* **4**, 22 (1996).
4. R. Förster *et al.*, *Cell* **87**, 1037 (1996).
5. M. Pinto *et al.*, *Biol. Cell. (Ivry sur Seine)* **47**, 323 (1983).
6. M. A. Costa de Beauregard, E. Pringault, S. Robine, D. Louvard, *EMBO J.* **14**, 409 (1995).
7. P. Kaoutzani *et al.*, *J. Clin. Invest.* **94**, 788 (1994).
8. Lymphoid tissue was dissociated between glass slides and placed in Dulbecco's mimimum essential medium (DMEM) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). Cell aggregates were removed by centrifugation (at 100 *g* for 3 min). The cell suspension was then pelleted (at 100 *g* for 10 min) and resuspended in culture medium (10^7 cells/ml).
9. T. T. MacDonald and P. B. Carter, *Immunology* **45**, 769 (1982).
10. E. Friederich *et al.*, *Cell* **70**, 81 (1992).
11. E. M. Danielsen, *Biochemistry* **33**, 1599 (1994).
12. Rabbit antiserum to villin (s1.135) and rabbit antiserum to SI were used at 1/500 dilution. Immunocytochemistry was done as described, with peroxidase-labeled donkey antibody to rabbit Ig (Amersham) as secondary antibody. The immunoperoxidase reaction was visualized on semithin sections embedded in Epon with diaminobenzidine (1 mg/ml) (Sigma) and 0.015% H_2O_2 for 15 min. Quantification of steady-state villin and SI expression was done on permeabilized cells: After incubation with specific antibodies, cells were incubated for 1 hour with ^{125}I–protein A (Amersham). The radioactivity in the filters was counted in a Packard counter. The nonspecific binding was quantified by incubation with ^{125}I–protein A alone. Protein immunoblots were done as described in (*27*).
13. S. Kernéis, A. Bogdanova, E. Colucci-Guyon, J. P. Kraehenbuhl, E. Pringault, *Gastroenterology* **110**, 515 (1996).
14. R. L. Owen and D. K. Bhalla, *Am. J. Anat.* **168**, 199 (1983).
15. T. C. Savidge, M. W. Smith, S. Mayel-Afshar, A. J. Collins, T. C. Freeman, *Pfluegers Arch. Eur. J. Physiol.* **428**, 391 (1994).
16. M. W. Smith, *J. Cell. Physiol.* **124**, 219 (1995).
17. T. C. Freeman, B. S. Bentsen, D. T. Thwaites, N. L. Simmons, *Pfluegers Arch. Eur. J. Physiol.* **430**, 394 (1995).
18. About 10^6 follicle cells in 100 μl were injected in the duodenal mucosa, between the pylorus and the first visible PP. The injection location was marked by a surgical thread. The injected duodenal segments were resected after 9 days, washed with phosphate-buffered saline, and either embedded in Tissue-Tek (Miles, Elkhart, IN) and snap frozen for immunocytochemistry or fixed in 2% glutaraldheyde in 0.1 M cacodylate buffer at pH 7.4 for light and electron microscopy.
19. S. Kernéis, A. Bogdanova, J. P. Kraehenbuhl, E. Pringault, unpublished data.
20. J. Pappo and T. H. Ermak, *Clin. Exp. Immunol.* **76**, 144 (1989).
21. FITC-conjugated latex beads (Molecular Probes, Eugene, OR; carboxylate-modified fluorospheres, 0.02 μm) were diluted in DMEM 2% fetal bovine serum (1.6×10^{10} beads/ml), placed in the apical compartment of the filter, incubated at 4°C, and switched at 37°C. Aliquots of the medium from the basolateral compartment were taken at different times. The bead numbers are given as arbitrary units that represent the number of fluorescent events per volume unit quantified with a FACScan flow cytometer. Each point represents the average value of three separate experiments.
22. M. Bens *et al.*, *Am. J. Physiol.* **270**, 1666 (1996).
23. T. C. Savidge and M. W. Smith, in *Advances in Mucosal Immunology*, J. Mestecky, Ed. (Plenum, New York, 1995), pp. 239–241.
24. J. P. Kraehenbuhl, unpublished data.
25. R. L. Owen, N. F. Pierce, R. T. Apple, W. C. Cray Jr., *J. Infect. Dis.* **153**, 1108 (1986).
26. Infection of epithelial cells was carried out essentially as described in (*28*). Bacterial suspension was inoculated in the apical compartment of the filter (10^8 bacteria) and incubated for 1 hour at 4° and 37°C. Transepithelial resistance was measured by a Millicell Ohmmeter (Millipore, Saint Quentin Yvelines, France) before and after the infection. Intracellular bacteria were counted after incubation of the filters with gentamycin (50 μg/ml). Epithelial cells were than lysed and the suspension was plated on colonization factor agar (CFA). We counted translocated bacteria by plating the medium from the basolateral compartment on CFA. Results are given as the number of CFU per square centimeter of confluent cells.
27. H. P. Hauri, E. E. Sterchi, D. Bienz, J. A. Fransen, A. Marxer, *J. Cell Biol.* **101**, 838 (1985).
28. W. N. Burnette, *Anal. Biochem.* **112**, 195 (1981).
29. P. Panigrahi, B. D. Tall, R. G. Russell, L. J. Detolla, J. G. J. Morris, *Infect. Immun.* **58**, 3415 (1990).
30. We thank M. R. Neutra and R. Golsteyn for suggestions and criticisms; G. Fischer, T. Laroche, and M. Allegrini for semithin sections, confocal laser scanning, and photographic work, respectively; G. Fischer, H. Ohayon, and P. Gounon for electron microscopy; and J. M. Fournier, D. Louvard, I. Chantret, H. P. Hauri, and B. Corthezy for providing *V. cholerae* 0:1 Ogawa and the antibody specific for O:1 serotype, villin, SI, Na^+-and K^+- dependent adenosine triphosphatase and (Na^+,K^+ ATPase), Na^+,K^+- ATPase the polymeric Ig receptor, respectively. Supported by the Swiss National Science Foundation, Sandoz, Zyma, Muschamp, the Ipsen and Singer Polignac Foundations, the Pasteur Institute, and INSERM.

18 February 1997; accepted 3 June 1997