

## SCIENCE ALERT

## Mid-life crisis for M cells

Kernéis S, Bogdanova A, Kraehenbuhl J-P, Pringault E. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* 1997;277:949-52.

**Abstract**

**The epithelium that lines the gut is impermeable to macromolecules and microorganisms, except in Peyer's patches (PP), 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.**

**Comment**

In the intestine, lymphoid nodules, individually or aggregated into Peyer's patches, are sites where antigen recognition begins and mucosal immune responses are initiated. M cells in the epithelium covering these lymphoid nodules are specially differentiated to take up and transport antigenic macromolecules and microorganisms from the lumen across the epithelial barrier, that otherwise restricts potential systemic pathogens to the lumen and prevents unregulated migration into tissues. M cells, enterocytes, goblet cells, and other epithelial cells lining the intestine and other mucosal surfaces are end stage cells, replaced from stem cells in an unending flow, that allows cells, physiologically infected by luminal microorganisms after emerging from the mouths of crypts, to be continually replaced by fresh recruits. Since M cells were first identified in humans in 1974,<sup>1</sup> a major limitation and challenge for investigators has been their inability to grow M cells in culture under controlled conditions where regulation of growth, development, and function could be delineated.

Lack of methods for in vitro investigation of lymphoid follicle epithelium, except in short term organ culture, has required deductive approaches to understanding factors which regulate M cell differentiation. Bye *et al* found that M cells already display detectable differences in enzymatic, cytoskeletal, and surface characteristics upon emerging from crypt mouths.<sup>2</sup> Remarkably, cells streaming from mouths of crypts surrounding intestinal lymphoid nodules show enterocyte characteristics when they flow from the sides of crypt walls next to villi, but on the opposite side of the same crypts, M cells originate from stem cells abutting lymphoid follicles. This observation generated the hypoth-

esis that lymphocytes or their intercellular mediators provide stimuli directing differentiation of intestinal epithelial stem cells.

A series of refreshingly direct yet technologically sophisticated in vitro and in vivo studies by Kernéis *et al* have provided new insights into the roles of intestinal lymphoid cells in the regulation of M cell differentiation and maturation. The hypothesis that lymphocytes or their products direct differentiation of intestinal crypt stem cells was tested by injecting Peyer's patch lymphoid cells beneath the epithelium of non-patch areas of mouse and rabbit intestine. Mucosal lymphoid nodules were generated with typical follicle associated epithelium containing functional M cells. Thus, the controlling stimuli for generation of intestinal lymphoid follicles seem to be factors produced by lymphoid cells aggregated in particular loci by homing receptors or other factors within intestinal vasculature rather than a genetically defined distribution of stem cells uniquely able to generate M cells.

Kernéis *et al* also developed an in vitro system for generating M cells from cultured human Caco-2 enterocyte cell lines, using dispersed murine Peyer's patch B and T lymphocytes lacking detectable macrophages or dendritic cells. When these lymphocytes were introduced into chambers separated by filters from confluent sheets of cultured enterocytes, they migrated into spaces between these cells, forming interepithelial pockets in enterocytes with disordered apical microvilli, characteristic of M cells. Alterations in brush border hydrolases typical of follicle epithelium also occurred in enterocytes without obvious lymphocyte contact, implicating soluble factors as well as direct contact in enterocyte differentiation. Interestingly, dissociated thymocytes did not induce formation of new mucosal lymphoid nodules.

Does this mean that differentiated enterocytes in vivo can undergo a mid-life career change into M cells? Not necessarily. Although each mammalian cell contains genetic code with the potential for expressing all the possibilities of any cell in the body, the lack of terminal differentiation which allows Caco-2 cells to replicate in culture more closely resembles that of crypt stem cells than of mature enterocytes. Identifying factors responsible for initiating and suppressing expression of intestinal stem cell differentiation genes in this system may also give clues to regulation of renewal of enterocytes in inflammatory conditions with increased numbers of interepithelial lymphocytes.

The ability of mouse cells to induce differentiation in human enterocytes suggests that both the inductive lymphocyte factors and corresponding epithelial receptors must be important enough to be conserved across species lines. Interestingly, murine lymphocyte stimulation of cultured human enterocytes induced not only structural features characteristic of M cells but also the ability to take up and transport *Vibrio cholerae*, which are non-invasive.

These studies point the way to future investigations of specific lymphocyte factors which induce M cell morphological and functional differentiation, information which might be exploited to increase uptake and transport at the

time of introduction of mucosal vaccines or to retard and block uptake during critical periods after administration of irradiation or radiomimetic immunosuppressive drugs, when patients are otherwise susceptible to systemic infection by endogenous intestinal organisms.

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- 1 Owen RL, Jones AL. Epithelial cell specialisation within Peyer's patches: An ultrastructural study of human intestinal lymphoid follicles. *Gastroenterology* 1974;**66**:189–203.
- 2 Bye WA, Allan CH, Trier JS. Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. *Gastroenterology* 1984;**86**:789–801.

## Call for Patients with Familial Pancreatic Disease: The EUROPAC Register

We are establishing a national UK register (EUROPAC) of families with hereditary pancreatitis, familial pancreatic cancer and where pancreatic cancer has occurred as part of a familial cancer syndrome. This collaboration in Liverpool is between the Department of Clinical Genetics ( Dr Ian Ellis) and the Academic Department of Surgery (Professor John Neoptolemos). The data and samples are collected by behalf of ESPAC (the European Study Group for Pancreatic Cancer), Professor Markus Büchler, Berne, and Professor Hans Beger, Ulm. The study will collaborate with Dr David Whitcomb of the Midwest Multicenter Pancreatitis study group in the United States. We aim to recruit families who are prepared to donate blood for DNA studies. We hope to gain a clearer understanding of the genetic relationship between hereditary pancreatitis and familial pancreatic cancer, and develop screening protocols for individuals at risk.

Hereditary pancreatitis is associated with a mutation in the recently identified cationic trypsinogen gene. This mutation renders the enzyme active within the pancreas, leading to autodigestion. Individuals with recurrent pancreatitis have a greatly increased risk of developing pancreatic cancer, and there is some evidence that DNA analysis of cells from pancreatic fluid may be valuable in detecting premalignant changes which can predict the development of pancreatic adenocarcinoma.

The criteria for inclusion in the study are as follows:

- Hereditary pancreatitis: Three relatives with chronic pancreatitis in the absence of ethanol dependence, hypercalcaemia, or an obstructive cause.
- Familial pancreatic cancer: Two first degree relatives with pancreatic adenocarcinoma. Three or more relatives with pancreatic ductal adenocarcinoma. Pancreatic ductal adenocarcinoma in any two relatives where the sum of their ages is less than 110 years.
- Other familial cancer syndromes: A single documented pancreatic ductal adenocarcinoma in any family with an established familial cancer syndrome—for example, BRCA2, FAMMM, A-T, HNPCC, or FAP.

If you know of any suitable families who may be interested in joining the study, please contact: Fiona McDonald, Clinical Genetics, Alder Hey Children's Hospital, Eaton Road, Liverpool L12 2AP. Tel: 0151 252 5905.

Thank you for your help.