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New targets in IBD: Crohn's disease. The NOD2 gene and Paneth cells.

Mutations in the MOD2 gene are strongly associated with increased risk of developing Crohn's disease of the ileum, which is part of the small intestine adjoining the colon. However, the mechanisms by which these mutations cause Crohn's disease remain unknown. Monocytes, which are circulating white blood cells that play a key role in protecting the body against bacteria express the highest levels of NOD2, although macrophages, which are derived from monocytes, express much lower levels. We recently showed that Paneth cells, which are specialised epithelial cells that form part of the lining of the small intestine, express the NOD2 gene at much higher level than macrophages. These results which we have submitted for publication, clearly show that Paneth cells are the major site of NOD2 expression locally in the intestine. A picture of Paneth cells expressing the NOD2 gene is shown in the figure on the right, where arrowheads indicate Paneth cells. NOD2 expression is demonstrated by brown staining, using a highly sensitive and specific technique called in situ hybridisation. There are many macrophages in the tissue surrounding the Paneth cells, which do not contain NOD2. This exciting discovery could explain why the mutations in the NOD2 gene are exclusively associated with Crohn's disease of the ileum, rather than other parts of the intestine, as Paneth cells are found only in the small intestine, and are most abundant in the ileum, while monocytes are ubiquitously distributed in the body.

Paneth cells comprise about a tenth of the cells in the crypts of the small intestine, from which all intestinal lining cells develop, and they are specialised for secreting proteins into the intestinal lumen. These proteins include a number of enzymes that digest bacteria, as well as many smaller proteins, called defensins, that kill bacteria by creating holes in their walls. In mice, defensins are essential for resistance to intestinal infection with bacteria such as *Salmonella typhimurium*. Paneth cells also participate in inflammation as they can produce mediators of inflammation, such as tumour necrosis factor α (TNF α), which is critically important in Crohn's disease. The importance of TNF α in Crohn's disease is illustrated by the powerful beneficial effect that antibodies to TNF α have in treating some forms of the disease. In addition although Paneth cells are normally absent from the large intestine, in IBD they appear for the first time in the inflamed tissues. We still do not know if this is simply an association with the inflammation or if it is linked mechanistically with the disease process.

The NOD2 protein probably functions by binding to an important component of bacterial cells called lipopolysaccharide or LPS. Monocytes respond to LPS through a molecule called toll-like receptor 4, or TLR4, while the mechanism by which Paneth cells respond to LPS is still unknown. We hypothesise the Paneth cells use NOD2 to interact with LPS, and therefore inheriting an abnormal form of NOD2 may have profound effects on how Paneth cells function, while monocytes can use

alternative pathways to compensate for the inherited defect. Our aim in this project is to test this hypothesis experimentally.

It is important to understand how the NOD2 protein functions in Paneth cells, as they are exclusively present in the intestine. So that the new therapies targeting Paneth cells will provide great specificity and thus potentially avoid side effects. Many current treatments for IBD suppress the immune system generally, while steroids, used to treat relapses of Crohn's disease, have widespread effects on body metabolism, hormonal balance, bone structure and mental function.

The experimental plan is to determine the NOD2 gene status in our patients with IBD and other illnesses, and then investigate the function of blood and intestinal cells from people who have inherited two normal copies of the NOD2 gene, one abnormal and one normal copy, or two abnormal copies. We will isolate monocytes from the blood, macrophages from biopsies of the small intestine (obtained during colonoscopy), and crypts, containing Paneth cells, also from biopsies. In addition, when patients have surgery to the small intestine, we will isolate macrophages and crypts from resected material that is no longer required for diagnosis.

We will measure the release of enzymes from Paneth cells and macrophages, in response to LPS and live bacteria. In particular, we will test the effect of a strain of bacteria called LF82, which was isolated from patients with Crohn's disease of the ileum. Professor Darfeuille-Michaud, who isolated these bacteria, has kindly provided them to us for these experiments.

The NOD2 gene may also be involved in regulating the death and survival of immune and Paneth cells, and in regulating the synthesis and release of inflammatory mediators. We will therefore also measure these aspects of cellular function, with the overall aim of comparing the effect of mutations in the NOD2 gene on response of Paneth cells with those of monocytes and macrophages.

We are requesting support for a salary for Dr Teresa Chalmers-Watson, who is a specialist registrar in Gastroenterology, and who will conduct the research as part of a post-graduate degree, and for essential reagents for the experiments. We are well placed to conduct this research as we run a large IBD clinic, have modern, high quality laboratories, and active collaborations with colleagues who are also working on trying to understand the causes and optimal treatment of IBD. In addition, this research project is unique in focussing on the function of Paneth cells, and we would therefore enjoy a competitive advantage in pursuing this exciting and novel line of research at this time.