

Dr Satish Keshav – Royal Free & University College, London

Grant awarded £85,000 (2 years)

Tissue-specific and circulating effectors in Crohn's disease

Exciting recent scientific advances in the genetics of inflammatory bowel disease (IBD) and in research into mechanisms by which white blood cells are attracted to the intestine have increased our understanding of the basis of Crohn's disease (CD). The NOD2 gene is mutated in some patients with CD of the small intestine (ileum), while a group of genes on chromosome 5, which encode protein messengers called cytokines that regulate immune and inflammatory processes, are associated with increased risk of all types of CD. It has also recently been shown that lymphocytes, which are white blood cells that are particularly relevant to IBD, are attracted to the intestine by a cytokine called TECK that is secreted by some epithelial cells that line the small intestine. TECK acts on a receptor, CCR9, which is expressed on the surface of almost all lymphocytes in the small intestine. Work on experimental animal models of IBD, pioneered by Professor Fiona Powrie, demonstrates that a subset of lymphocytes, called TH1 cells, can cause intestinal inflammation, while a separate subset, called regulatory lymphocytes, or Treg cells, can prevent or cure disease. The two cell subsets (TH1 and Treg) secrete characteristic cytokines that have largely opposing effects. A small number of lymphocytes circulating in the blood express CCR9 on their surface, and probably enter the small intestine, where they could interact with the lining epithelial cells. These CCR9-positive cells comprise a mixture of TH1 and Treg cells, and they are more numerous in patients with CD. It is not known whether the ratio of TH1 to Treg cells changes in disease, or with the use of immunosuppressive treatments such as azathioprine and mercaptopurine that are prescribed to achieve better disease control, and this is one aim of our proposed project.

In a preliminary study where we blocked the recruitment of immune cells to the intestine in a model of Crohn's disease, we noticed interesting alterations in the epithelial cells that line the intestine, particularly Paneth cells, associated with inflammation and treatment. Paneth cells are specialised epithelial cells that play a role in defence against bacteria, and we recently showed that they are the specific site of NOD2 gene expression in the intestine. The number of Paneth cells was reduced in the inflamed intestine, while in the treated group, where infiltration by white blood cells was markedly reduced, the number of Paneth cells was increased, and each Paneth cell contained many more granules.

Representative microscopic pictures are shown in the figure below. The white arrows point to pink stained Paneth cell granules, showing a normal number in two adjacent crypts in the control, reduced numbers in a single crypt with inflammation, and increased numbers in an enlarged crypt with treatment. Goblet cell numbers were also increased with inflammation, and the arrowhead points to a goblet cell. A white line has been drawn around the base of each crypt for clarity. These data highlight the importance in IBD of interactions between circulating blood cells and tissue specific epithelial cells in the intestine. The purpose of this project is to investigate aspects of this interaction, concentrating on circulating CCR9-positive lymphocytes and their effects on intestinal epithelial cells in tissue culture. We propose to enrol patients with CD and ulcerative colitis (UC), and those without IBD, from our gastroenterology and IBD clinics. We will

obtain blood samples at the same time as routine clinical samples are collected and intestinal biopsy samples at the time of colonoscopy, from patients who are undergoing this procedure as part of their clinical care, with full informed consent of the patients. Details of the patients' clinical condition and the medications they are using will be recorded. The Royal Free Hospital's ethics committee will review the study protocol. One part of the project involves using well-established techniques such as flow cytometry and assays to measure the secretion of cytokines in order to determine the ratio of circulating intestine specific (i.e.CCR9-expressing) Treg cells to TH1 cells. We will correlate this information with the clinical state of patients, and test our hypothesis that an increased ratio of Treg cells to TH1 cells is associated with reduced inflammation. In addition, we will determine the effect of concomitant treatment with immunosuppressive medications such as azathioprine or mercaptopurine on the ratio of Treg to TH1 cells. This could eventually allow us to develop a simple blood test to monitor the efficacy of such treatment. We will also determine the effect of culturing lymphocytes directly with biopsy tissue from patients.