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Grant awarded £76,775 (2 Years)

Gene-environmental interactions in Inflammatory Bowel Disease: the effects of NOD-2 mutations and nicotine on NF-kB activation

The incidence of Inflammatory Bowel disease (IBD) is increasing in young people in the UK, affecting their growth, education, social well being and employment. Inflammation in bowel is under control of different cellular processes, which may be influenced by the genetic makeup of individuals, or more controversially, environmental factors such as the gut bacteria present in the bowel or the smoking habits of the individual. One key cellular process is the activation of a factor called NF-kB. NF-kB is involved in controlling the way cells respond to external stimuli (e.g. bacteria). Activation of NF-kB can lead to increased inflammation, and is known to be increased in IBD, especially Crohn's disease (CD).

A gene has been identified which codes for a protein (NOD-2) which is also involved in the immune response to bacteria and acts via NF-kB. Recently, it has been found that several mutations in this NOD-2 gene are more common in patients with CD than in patients with ulcerative colitis (UC) or the normal population. Preliminary evidence indicates that these mutations are also more often found in CD patients with ilea disease. These mutations produce abnormal NOD-2 proteins, but the function of these abnormal proteins have not been verified. Smoking is known to have an influence in IBD as it increases the severity of CD but is protective against UC. What is not clear, is how smoking affects the bowel to give such different effects in IBD. Although it is known that there are many compounds in cigarette smoke, previous studies on smoking have focused on nicotine. Some studies have used animal models and the results are not very consistent, but there is evidence that nicotine does affect the cells involved in immune responses. In UC, nicotine therapy has had some limited success, but the way in which it acts is still unclear. In CD, patients improve clinically when they cease smoking. However, it is not known whether nicotine supplements (e.g. patches, gum, lozenges) could be used to help patients give up smoking, or whether it would increase the inflammation in their bowel, thus making their disease worse.

This project seeks to investigate the interactions between the genetic and environmental influences in IBD, concentrating particularly on the NOD-2 gene mutations and nicotine and how they both influence NF-kB activation, either separately or together. We have preliminary data, from colorectal cancer cell lines to suggest that nicotine does directly influence NF-kB and may affect the cellular responses to bacteria.

We have identified IBD patients with and without the NOD-2 mutations. Blood samples will be taken, cells isolated and stimulated with nicotine at different concentrations or bacterial products to assess whether nicotine activates NF-kB in the same way as bacteria. Nicotine and bacterial products will also be added to the cells together to investigate whether nicotine changes the

immune responses to bacteria. NF-kB activity will be measured by extracting the proteins from the cells and looking for specific proteins involved in NF-kB activation. Other markers of inflammation, which are produced by the cells, will be measured. Responses from CD patients with and without NOD-2 mutations will be compared, as will responses from CD and UC patients without NOD-2 mutations. Responses from healthy controls will be measured in blood samples obtained from volunteers or from the Blood Transfusion Service and the NOD-2 genes will be assessed for mutations.

From the same group of patients, we wish to obtain ileal or colonic biopsies during routine follow-up colonoscopy, or resection specimens at surgery. In similar experiments to those using the blood samples, cells will be isolated from the biopsies and stimulated with nicotine and bacterial products, either separately or together. As outlined above, NF-kB activation will be measured by extracting proteins and identifying the presence or absence of specific proteins. This would give information about how different cell types in the gut respond to nicotine separately. In addition, we will culture whole colonic biopsies, without separating the cells, with nicotine and bacterial products. Again, inflammation will be assessed by measuring secreted inflammatory mediators. The biopsies will be fixed and processed. Sections of tissues will be stained to identify which cells contain activated NF-kB. This would give information about how the different cells respond to nicotine when they are in contact with each other, as they are in the body, and whether this contact changes their responses.

These experiments will provide important information on the relationship between genetic makeup of a patient and the mechanisms of development of IB. In addition, these experiments will indicate whether nicotine can be used to help patients with CD stop smoking.