

Title of project:

Macrophages from patients with Crohn's disease have an impaired response to bacteria

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Lay summary

Previous work by our group has suggested that patients with Crohn's disease may have impaired function of macrophages – one of the white cells that circulate in the blood and which migrate into the tissues to help heal inflammation and to protect us from the harmful effects of bacteria.

With the assistance of the grant from NACC, we have been studying how bacteria interact with the macrophages isolated from patients with Crohn's disease or ulcerative colitis and compared the results with those obtained using macrophages from healthy individuals. We have stimulated these cells with heat-killed bacteria (specifically a bacterium known as *E. coli*) and measured the release of some of the chemicals (cytokines) that are important for establishing inflammation. We have shown that cells obtained from patients with Crohn's disease release significantly less of these molecules than do cells from healthy individuals or even cells from patients with ulcerative colitis. Thus, we needed to find out why cells from Crohn's disease patients were different from cells derived from the other two groups. We know that chemical substances within the walls of bacteria can stimulate macrophages by combining with a series of receptors on the surface of the macrophages – these are known as Toll-like receptors (TLRs). We hypothesised that the impaired macrophage response to bacterial stimulation might reflect altered TLR function. Therefore, we stimulated macrophages with purified bacterial molecules which are specific for the individual TLRs. So far, we have tested those bacterial molecules that combine with either TLR2 or TLR4 and have shown impaired responses in Crohn's disease when macrophages are stimulated through TLR2, but normal responses when stimulated through TLR4. We are in the process of testing all the other TLRs to determine whether the abnormal response seen with TLR2 is specific to that receptor.

While these experiments are being completed, we have studied the density of TLR2 on the cell surface of the macrophages and the sequence of events that occur within the cell leading to the release of the inflammatory chemicals (cytokines) which is triggered by a bacterial-derived compound binding to the TLR2. We have been unable to demonstrate any abnormalities in macrophages from patients with Crohn's disease so far. Finally, we have examined the structure of the gene that codes for the TLR2 protein. We have done this using DNA from patients with Crohn's disease whose macrophages showed the most marked impairment to bacterial stimulation. We have found a novel mutation which seems to be present in some healthy individuals, but whether this has significance for Crohn's disease is not yet clear.

We would like to thank NACC for funding these studies over the last year which has allowed us to obtain a better understanding of macrophage function in patients with Crohn's disease. The results of these studies are currently being prepared for publication.