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Lay Summary

How do cells defend their cytosol against invading bacteria and could failure cause inflammatory bowel disease?

Inflammatory bowel disease is caused by a failed balance between our immune system and the bacteria inhabiting our gut. Genetic studies of patients with Crohn's disease have identified gene variants that make their owners more likely to develop the disease. As expected, many of these susceptibility genes function in our immune system. For most susceptibility genes, however, it is not known how their malfunction triggers inflammatory bowel disease. Interestingly, variants of two genes (called NOD2 and ATG16L1) act inside cells to destroy bacteria that invade the interior of our cells. This finding suggests that in our digestive tract bacteria are routinely attempting to enter the cytosol of cells and that a failure to destroy these cytosolic bacteria will result in sustained inflammation, which eventually may develop into inflammatory bowel disease.

The defence against cytosolic bacteria is probably the least understood of all immune mechanisms. We know that in a process called autophagy invading bacteria become surrounded by membranes before being delivered to lysosomes, a cellular compartment highly competent in killing bacteria. Cells often decide which 'object' should be taken up into an autophagosome by first marking it with ubiquitin, a tiny protein that cells can chemically attach to other proteins. When John Brumell and his colleagues discovered in 2004 that invading bacteria become decorated with ubiquitin a race began to discover the cellular receptor that recognizes these ubiquitin-coated bacteria. My group discovered that NDP52 is this long sought-after player. In the absence of NDP52, cells fail to deliver the ubiquitin-coated bacteria efficiently into autophagosomes and are therefore unable to limit the multiplication of bacteria that have invaded their cytosol.

NDP52 and the machinery that decorates invading bacteria with ubiquitin, if functioning efficiently, may therefore help to avoid inflammatory bowel disease. I am now proposing to identify the proteins that mark bacteria with ubiquitin and to discover how NDP52 achieves the remarkable task of delivering ubiquitin-coated bacteria for destruction.

The process of attaching ubiquitin to other proteins ("ubiquitylation") is known in great detail. It requires three proteins that act sequentially – called E1, E2, and E3. Almost all ubiquitylation is performed by just one E1. However, identifying which of the several dozen E2s and several hundred E3s are responsible to specifically decorate bacteria is a formidable task. My group has developed a reporter cell line to quantify the number of ubiquitin-decorated bacteria. We will use a method called siRNA interference to remove individual E2s and E3s from our reporter cells. Using an automated microscope and our novel reporter cell line we will identify which E2 and E3 are essential to mark bacteria for destruction. This knowledge will allow us to test whether these proteins contribute to inflammatory bowel disease.

To understand how NDP52 delivers bacteria for destruction we will delete the gene for NDP52 from human cells that we can grow in tissue culture. Such work is normally performed in mice in a process that requires about two years of intense work. My group has developed a much faster technique to delete genes from human cells, thereby allowing the study of human rather than murine cells and reducing the requirements for experiments with animals. We will use our novel technique to delete NDP52 from human gut cells to investigate how cells without NDP52 cope with bacteria that invade their cytosol. These cells will also allow us to study variants of NDP52, for example variants with an altered ability to detect ubiquitin-decorated bacteria, in order to determine the mechanism how NDP52 delivers bacteria for destruction.