G. NON TECHNICAL SUMMARY (NTS)

Project title: Understanding gene – environment interaction in inflammatory bowel disease
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: YES
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants: YES
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes: NO

   (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b): NO
   (d) protection of the natural environment in the interests of the health or welfare of man or animals: NO
   (e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work: NO
   (f) higher education or training for the acquisition, maintenance or improvement of vocational skills: NO
   (g) forensic inquiries: NO

Keywords:
Inflammatory bowel disease, Crohn's disease, arthritis, Infection, Tumours

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
Crohn's disease and ulcerative colitis are the two major forms of inflammatory bowel disease. These are chronic, debilitating diseases that usually occur early in life and lead to severe inflammation of the intestinal tract. These diseases ruin lives, and can also lead to cancer. The UK is amongst the countries with the highest risk for this disease, it is estimated that -1 in 200 individuals is affected. Risk for these diseases runs in families, and the genes responsible for this risk have been largely discovered over the last decade. However, the genes in themselves do not cause disease, but it requires other – yet unknown – factors that trigger disease. Unfortunately, even the function of most of the risk genes and how they would lead to disease, remain mostly unknown. Here we aim to study the function of important risk genes to discover the 'pathways' and cells in the body that are engaged that lead to disease. We will also aim to use this knowledge to identify and study environmental factors that may trigger disease in an individual who carries 'risk genes'. In our intestine, we carry an enormous number of bacteria and other microbes, and they seem to be the target, but possibly also a trigger, of a misguided immune response that is typical of inflammatory bowel disease. We will hence study the role of the microbiota and how this affects the triggering of disease.

In summary, we want to understand how IBD risk genes function, and how they interact with environmental triggers. This will not only increase our understanding of the mechanisms that cause...
disease, but may identify novel targets for treatment that could subsequently be developed into new medicines.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?
We hope that our investigations will identify new treatment targets for inflammatory bowel disease, and possibly also for other immune-mediated diseases. We hope to begin to understand what triggers may set off intestinal inflammation. This is very important as this disease has become way more common in the Western World over the last decades, and is now also picking up speed in other parts of the world such as Asia.

What types and approximate numbers of animals do you expect to use and over what period of time?
Over 5 years, we will study ~24000 mice. This amounts to 1.3 mouse per day per researcher. We have a strong track record of major discoveries in this field over > 15 years, all on the background of adhering to best practice and highest ethical standards. This includes experimental design principles where we apply highest standards including random allocation to experimental groups, and blinded analysis – all meant to avoid bias introduced by investigators.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?
The vast majority of genetically-altered mouse lines we study develop entirely normally. They will typically undergo a tissue biopsy from the ear to determine their genotype, and for a large number of animals this will be the only intervention and no adverse effects are expected from that. Mice may be fed specific diets (e.g. high-fat diets), or they may receive antibiotics to modulate the bacterial flora in their intestine – interventions that are typically well tolerated and only few adverse effects would be expected that we will monitor for (e.g. diarrhoea). In a small proportion of mice we will induce colitis, infection, arthritis, or tumours. This can be associated with diarrhoea, weight loss, and compromised overall well-being. No more than 10% of animals studied in this project license are expected to show moderate clinical signs such as piloerection. Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are monitored on a regular basis to detect any sign of distress or suffering. Analgesic agents will be administered as required. In the event of complications, or at the end of the experiment, animals will be killed by a schedule 1 method.

Application of the 3Rs
Replacement:
The number of mice for this project may seem relatively high, although it is ~1.4 mouse per day per researcher. This is due to the lack of reliable in vitro models that capture the complexity of the intestinal tract: there is a myriad of microbes in faeces, and a thin layer of mucus and cells that separates them from the body’s intestinal cells – which, for example, contain the body’s largest accumulation of immune cells. A pathologic immune response to these microbes is a hallmark of inflammatory bowel disease. There are absolutely no in vitro models available that would even remotely capture the complexity of the organ and therefore we have to rely on mouse models.

Reduction:
We are using a wide array of sophisticated in vitro experiments, including various types of cells and cell lines, and all sorts of molecular techniques to predict the establish biological mechanisms. This allows us to make predictions and to prioritise those mechanisms that need to be studied in mice in vivo.

When designing experiments, we perform statistical analysis to determine the minimum number of
mice that are required to perform an informative experiment. We also always aim to maximise the information we can gather from every single mouse.

Refinement:
Animals are housed according to the best recommendations in an appropriate and enriched environment. By performing pilot studies and choosing well-established protocols based on extensive previous experience, we minimise the unknown effects on the mice and hence pain, distress and suffering. We very frequently monitor animal behaviour and well-being to detect any upcoming problem at an early stage.

For one of the most important genetic risk factor of inflammatory bowel disease (which is carried by ~two thirds of all patients), over the last years we have developed a model of small intestinal inflammation that spontaneously develops, and which is not associated with any clinical disease or suffering of the animal. It is purely visible under the microscope. This is a major advance, and we have validated that this closely resembles human Crohn's disease.