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SECTION 1

THE MOLECULAR CONTROLS OF RESOLUTION OF INFLAMMATION: WHAT CAN WE LEARN FROM ZEBRAFISH?

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WINNING ABSTRACT: Although we are separated from zebrafish by 160 million years of evolution, we share many features of the innate and adaptive immune systems. In addition, we can manipulate the genome of zebrafish, and observe the effects on inflammation *in vivo* as they are transparent in their larval stages. This has exciting implications for the study of inflammatory diseases.

We have established a model of inflammation in the zebrafish tail, in which caspase dependent cell death is required for resolution. For example, addition of the pan-caspase inhibitor zVD added at 4 hours after tailfin injury increases the number of neutrophils present from 6.0+/-1.0 to 28.9+/- 3.3 (mean +/- s.e.m. $p<0.001$ $n=3$).

The transparency of the larvae makes these an ideal model for the study of *in vivo* inflammation, and we have generated fluorescent systems for the easy visualisation of neutrophilic inflammation and resolution *in vivo*.

We are also performing an unbiased forward genetic screen for mutants with defective resolution of inflammation, and to date have identified 38 putative mutants. These techniques allow new approaches to understanding the molecular controls of inflammation resolution.

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MY JOB AND THE UNIT IN WHICH I WORK

I am an MRC Clinician Scientist, Senior Clinical Lecturer and Honorary Consultant Respiratory Physician working at the University of Sheffield School of Medicine and Biomedical Sciences (Sheffield, UK). I am currently establishing a research group to extend my studies of the role of neutrophils in inflammatory lung disease into a novel model of inflammation in the zebrafish, *Danio rerio*. To that end, I have initiated a very successful collaboration with Prof. Philip Ingham, FRS, in the MRC Centre for Developmental and Biomedical Genetics within the University. I have laboratory space within the Centre, where we need access to both the aquaria and the expertise available. When I am not working in the laboratory, I spend my time at the Royal Hallamshire Hospital, where I perform clinical sessions and other duties within the Medical School. I participate in the rota to provide cover to the in-patient respiratory service and perform general medical tasks during this time. I also cover clinics and bronchoscopy sessions from time to time. I am subspeciality lead for pleural disease in Sheffield and am currently working to establish a local anaesthetic thoracoscopy service within the Sheffield hospitals.

MY WINNING POSTER AS PART OF MY RESEARCH

My research interest in the molecular mechanisms underlying inflammatory lung disease has come from my clinical interest in this broad group of diseases. Since beginning my PhD studies with Prof. Moira Whyte in 1998, I have been interested in the molecular controls of neutrophil lifespan and how this relates to the resolution of inflammation. We believe that the process is controlled by a series of molecular events, leading to the death of neutrophils by apoptosis. Thus, regulators of apoptosis regulate inflammation. My PhD thesis explored the role of a family of death receptors on the neutrophil surface [1, 2] and their intracellular signalling pathways [3], which may be involved in regulating inflammation *in vivo*, but also give the potential to target neutrophil lifespan therapeutically. However, work on human neutrophils is limited by their genetic intractability and I had been seeking alternative model systems for some time. We are very fortunate in Sheffield to have a world-leading zebrafish group led by Prof. Philip Ingham. In collaboration with Prof. Ingham, I have established a model of resolving inflammation, in which individual neutrophils can be visualised easily *in vivo* in real time [4]. We have established that inhibition of apoptosis proteases

(caspases) causes delay of resolution of inflammation in this model. The real strengths of zebrafish models are the ability to perform forward (unbiased) and reverse (candidate gene) genetic screens for the molecules involved in inflammation. These screens are underway and I hope to be reporting their results within the next 1–2 yrs.

MY RESEARCH AS PART OF MY WORKING GROUP/ RESEARCH TEAM

The Academic Unit of Respiratory Medicine (AURM) is headed by Prof. Moira Whyte. Other principal investigators in the AURM are Prof. Ian Sabroe, Dr David Dockrell, Dr Colin Bingle, Dr Sarah Walmsley and myself. My own interests in the molecular controls of the resolution of inflammation are mirrored by many within the group. The uniting theme of the research group is the interaction of the host innate immune system with pathogens. Ian Sabroe is a Medical Research Council Senior Clinical Fellow and has diverse interests, but his main research topic is the influence of signalling through Toll-like receptors on neutrophil biology. David Dockrell is a Wellcome Trust Senior Clinical Fellow and is interested in the effect on the biology of the macrophage of interactions with pathogens, particularly *Streptococcus pneumoniae*. Colin Bingle is Senior Lecturer and, among his widespread interests, the novel peptides involved in the innate immune response to pathogens are an emerging focus for his work. Sarah Walmsley is a Wellcome Trust Intermediate Fellow, with an interest in the role of hypoxic signalling in regulating inflammatory neutrophil biology. Many of these programmes of work study mechanisms of neutrophil apoptosis, and there is much collaboration and cross-fertilisation of ideas. We use a number of models to study these processes, including cell lines, primary human neutrophils (either in culture alone or with other relevant cells), and *in vivo* models including the zebrafish.

There are other respiratory research programmes within respiratory medicine in Sheffield, notably those of David Fishwick and Christopher Barber, who study occupational lung diseases and work closely with the Health and Safety Laboratories in Buxton.

THE IMPACT OF MY WORK ON CLINICAL OR RESEARCH PRACTICE

Inflammatory disease is in need of new treatments. The treatments we have are poorly effective for many of the conditions they are used for and are beset by side-effects. Understanding how inflammation resolves would allow us to understand how it might fail to do so in inflammatory disease, and thus identify targets for the development of novel classes of drug. My model will allow real-time visualisation of inflammation as it occurs and also permit genetic manipulations, including forward genetic screens. This will allow us to come to a better understanding of the genetic controls of the resolution of inflammation. It is my hope that this will allow identification of critical control points, where small molecules may be able to target inhibition of normal resolution allowing it to proceed unhindered. In fact, zebrafish models such as this are ideal for screening for novel drugs targeting the immune response. I would hope that this model will inform many aspects of inflammatory cell biology and lead to a better understanding of this essential physiological process.

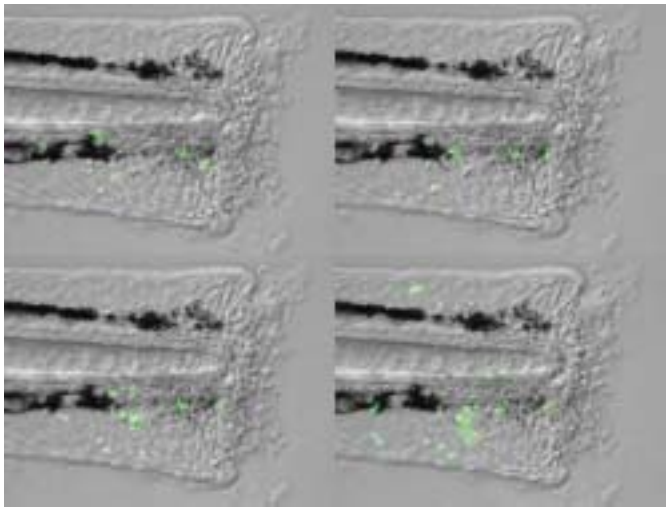

FIGURE 1. Following injury to the tail fin of a transgenic zebrafish larva at 3 days post-fertilisation, green fluorescent protein-positive fluorescent neutrophils can be seen accumulating at the site of injury. The images shown are taken 15 min apart and read from left to right and top to bottom.

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