

Capturing Signaling Events in the Immune System *in Situ*

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*Dr. Harnett is Professor of Immune Signaling at the Division of Immunology, Infection and Inflammation at the University of Glasgow's Biomedical Research Centre. Her research career has been in the field of Immune Cell Signaling, with initial interests in the mechanisms underlying G-protein regulation of neutrophil degranulation and respiratory burst dysfunction in immune deficiency. She then became interested in the nascent field of lymphocyte signaling at the National Institute for Medical Research, London, where she focused on identifying the key regulatory elements involved both in coupling the B cell antigen receptor (BCR) to lipid signaling pathways under mitogenic conditions and in their desensitization during negative feedback inhibition by immune complexes. Returning to the University of Glasgow, she established a group to study the signaling mechanisms underlying development of the immune response. This research has primarily focused on dissecting the differential signaling mechanisms associated with functional maturation of the immune response and its evasion by pathogens in order to identify novel targets for therapeutic intervention in autoimmune and allergic inflammatory disease. Over the last five years a particular focus has been to translate the analysis of intracellular signaling mechanisms from the test tube to the *in situ* physiological environment of the immune response in animal models.*

Abstract

Understanding the molecular mechanisms and cellular interactions that regulate both the induction and the phenotype of immune responses is central to the development of safe, efficacious therapies to combat infections and inflammatory disorders. Until recently, however, it has not been possible to analyze physiologically relevant interactions *in situ*, as the technology has not been available to directly visualize and functionally correlate the key molecular and cellular events underpinning immunity and tolerance in the intact immune system. Recent advances in quantitative imaging, such as laser scanning cytometry (LSC) and new methodologies in the analysis of cell signaling, now allow analysis of such signaling and functional events *in situ*. Indeed, we have used these approaches to show that the distinct functional outcomes of priming and tolerance are associated with inverse signatures of Erk and Rap1 GTPase signaling. Thus, our analysis suggests that the maintenance of tolerance of individual antigen-specific T cells *in vivo* may reflect the recruitment of up-regulated Rap1 to the immune synapse, potentially resulting in sequestration of Raf-1 and uncoupling of the TcR from the RasERKMAPKinase cascade and consequent cell-cycle progression and clonal expansion. Our current work in this area focuses on the role of E3 ligases in regulating these differential signals. Whilst breaking of tolerance can lead to autoimmune disease, inappropriate priming, such as that observed in allergy, can also result in inflammatory pathology. We have therefore utilized LSC to characterize key molecular and cellular events occurring *in situ* that underpin development and polarization of such aberrant autoimmune and allergic inflammatory responses. In addition, by exploiting the anti-inflammatory therapeutic potential of the filarial nematode-derived product, ES-62, we have used this approach to identify potential novel and clinically relevant therapeutic targets.