Mechanisms of Steroid Action and Resistance in Inflammation and Disease

A special section consisting of proceedings from the EUROSTERONE Meeting Dublin, Ireland, 13–14 September 2002

FOREWORD

The conference 'Mechanisms of Steroid Action and Resistance in Inflammation and Disease' was held in Trinity College Dublin in September 2003 and was the final thematic conference held under the auspices of the EUROSTERONE thematic network.

EUROSTERONE was funded under the European Union's Framework V programme, and aimed to increase collaboration among researchers in the field of steroid biology, to standardise research protocols and facilitate access to reagents. Another important remit of the network was to widely disseminate research findings by means of thematic conferences.

This was one of a series of conferences held which focussed on different aspects of steroid action. Previous meetings included 'Nuclear Receptors in the Brain' held in Oegstgeest, The Netherlands, 'Nuclear Receptors and their role in the Regulation of Inflammation' held in Huddinge, Sweden, and 'Steroid Signalling: New Frontiers' held in Edinburgh, UK. 'Mechanisms of Steroid Action and Resistance in Inflammation and Disease' sought to build on this series of conferences and explore the pathophysiological aspects of response to steroid signalling. Steroid-based regimens represent important treatments and indeed may be the treatments of choice in many chronic inflammatory conditions, including rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and asthma as well as cancers such as leukaemia. However steroid refractory disease is associated to a greater or lesser extent with each of these conditions and can impose a significant health burden – for example, steroid-resistant asthma patients, while only 5% of the total, account for 50% of the health care costs associated with disease.

The meeting consisted of three sessions designed to explore refractory conditions beginning with an update on steroid signalling and control of gene expression. This was followed by a session dealing with 'mechanisms of resistance to steroids'. The final session concentrated on 'steroid resistance and inflammatory disease' with a particular emphasis on asthma, but also RA and IBD. This special section in the *Journal of Endocrinology* features a number of articles drawn from the conference.

Steroids and the regulation of gene expression

The first session of the conference dealt with novel aspects of steroid signalling. Dr Andy Clark (Imperial College, London) discussed the role of glucocorticoids (GCs) in regulating the stability of mRNA's encoding proinflammatory proteins, in particular, the dual specificity phosphatase, MAPK phosphatase-1 (MKP-1). His group and others have shown that MKP-1 is induced by

dexamethasone and potentially inactivates p38. The p38 signalling pathway is important in regulating the stability of proinflammatory mRNAs including TNF- α , Cox-2 and IL-6, among others. Inhibition of p38 leads to the destabilisation of these mRNAs and the attenuation of the inflammatory response. Such post-transcriptional mechanisms at least partially explain their ability to regulate gene function long after the inducing stimulus and adds to their already formidable array of known functions designed to influence gene expression.

The activity of glucocorticoids in regulating signalling of Toll-like receptors (TLRs) was explored by Dr Paul Moynagh (University College Dublin). TLRs share considerable homology with the IL-1 receptor, in particular the intracellular Toll/IL-1R domain. In consequence, TLRs engage the same signalling pathways as IL-1 leading to activation of a familiar cast of pro-inflammatory transcription activators (e.g. NF κ B, AP-1). MAP kinase pathways (including p38) are also activated by TLRs and are targets for inhibition by glucocorticoids. GC are thus likely to exercise profound repressive effects on the innate immune system with downstream fallout for adaptive immunity and inflammation.

Dr Onno Meijer (University of Leiden) delivered an overview on the tissue-specific actions of GC using examples from the brain. Tissue specific effects depend on a number of factors including ligand availability, receptor diversity, the presence of cofactors and the transcriptional permissiveness, or otherwise, of potential DNA binding sites (as influenced by chromatin structure for instance). Many of these factors can vary considerably leading to very different actions by GC in neighbouring cells. Thus any cell-specific response corresponds to a non-responsive state in other cells and knowledge of these mechanisms could help in elucidating the mechanisms underlying GC refractory conditions.

Mechanisms of steroid resistance

Introducing this section, Dr Esther Sternberg (National Institute of Mental Health, Bethesda) gave an overview on the role of the hypothalamic–pituitary–adrenal (HPA) axis in susceptibility and resistance to inflammatory diseases. HPA hormones released in response to a variety of stresses suppress the production of GCs leaving the host vulnerable to inflammatory conditions if the situation persists. Dr Sternberg described genetic studies in mice which have identified a chromosomal region controlling the HPA axis and inflammation. Candidate gene analysis has so far ruled out CRH-R1 and ACE as the culprits. The discovery of mutations affecting GR β stability in RA was discussed as was the prospects for a CRH antagonist in treating inflammation in an RA model.

Dr Ann-Charlotte Wikström (Karolinska Institutet) dealt in detail with the influence of GC receptor (GR)–interacting proteins on GC responsiveness. The GR is capable of directly interacting with many other proteins and much of its influence on cellular behaviour derives from this capacity, as well as through transcriptional regulation. Using a proteomic approach Dr Wikström's group has demonstrated the interaction of GR with several interesting proteins including the adaptor protein 14–3–3, as well as Raf-1 under physiological conditions, indicating another example of direct crosstalk between GC and MAPK signalling cascades. Of note, they also demonstrate an interaction of non-liganded GR with NF κ B and I κ B α , indicating an even closer relationship between these proteins than had hitherto been suggested.

The specific example of resistance to glucocorticoid-induced apoptosis in lymphoblastic leukaemia, gave rise to a thought-provoking review by Dr Reinhard Kofler (University of Innsbruck). Dr Kofler emphasised the multifaceted nature of steroid resistance which can be reversible or not and dependent

on GC concentration or all-or-nothing, considerations which can have an impact on therapeutic decisions. He discussed the influence of variants of the GR and altered GR expression on steroid insensitivity. He went on to describe mechanisms downstream of the GR such as induction of survival pathways (e.g. IL-6) and the influence of anti-apoptotic systems (e.g. the Bcl-2 rheostat) which may prevent steroid signals from being executed.

Developing the focus on apoptosis, Dr Ian Dransfield (University of Edinburgh) spoke on the influence of GC in granulocyte apoptosis and macrophage activity as an important component in the resolution of inflammation. This critical process is responsive to physiologically relevant concentrations of GC which, for example, have opposite effects on eosinophils and neutrophils. Failure to clear these cells can lead to necrosis and the release of pro-inflammatory mediators further aggravating the inflammatory response. Monocyte differentiation is also subject to the effects of GC. Thus impairment of these mechanisms could exacerbate chronic inflammatory conditions and awareness of their impact may allow for improved patient management.

Steroid resistance and inflammatory disease

This session concentrated on what is known regarding steroid resistance in inflammatory diseases particularly asthma, RA and IBD. Drs Ian Adcock (Imperial College, London) and Stephen Lane (AMANCH, Dublin) have presented a joint paper summarising their separate lectures on the subject of GC resistance in asthma. As stated above, steroid insensitivity affects a small proportion of the patient population who represent a continuum from those requiring high doses to patients who are practically insensitive to GC leading to a pernicious condition that can be life threatening. The authors presented a comprehensive review of the molecular basis of inflammation in asthma and what is known regarding GC refractory asthma. Interestingly, they presented evidence that p38 may influence GC binding of the GR and that this may be significant in a proportion of severe asthma cases, as well as exploring the interaction of AP-1 and the GR in some detail.

Dr Garry Walsh (University of Aberdeen) revisited the subject of apoptosis in the resolution of inflammation with particular emphasis on asthma. Eosinophils are thought to be the major, pro-inflammatory, cellular player in asthma, and the question of their persistence in the airways may be due in part, to an imbalance of survival-enhancing factors (such as IL-3, -5, -13 among others) compared with apoptotic-inducing factors (e.g. CD95, -45, -69). The role of GC in promoting cell clearance by phagocytosis was discussed as was the possibility that defects in caspase-induced apoptosis may underly GC insensitivity in some cases.

Dr John Baugh (University College Dublin) reviewed the evidence that macrophage migration inhibitory factor (MIF) may play a pivotal role in regulating the magnitude of immune and inflammatory responses. Despite its name, MIF is a key component in the HPA axis with systemic pro-inflammatory actions affecting both the adaptive and innate immune systems. It is interesting to note that eosinophils are a significant source of MIF and MIF can counter the suppressive effects of GC. Dr Baugh described the identification of a polymorphism of the MIF promoter which correlates with altered MIF production. This seems to be associated with variable outcome in RA and investigations are ongoing in other diseases.

Changing the focus to inflammatory bowel disease, Dr Dermot Kelleher (Trinity College Dublin) described what is known of molecular mechanisms of GC resistance with particular emphasis on the P-glycoprotein (Pgp-170 encoded by MDR1/ABCB1). This protein has been shown to be overexpressed

on both T-cells and intestinal epithelial cells in both Crohn's disease (CD) patients and patients with ulcerative colitis (UC). Recently MDR1 polymorphisms have been reported to be associated with intractable UC but not CD. Thus it appears that blockage of Pgp-170 function or use of GCs which are not Pgp substrates may improve the treatment of these patients, an hypothesis that remains to be proven definitively.

Rounding off the conference, Dr Ian Chikanza (St. Bartholomews & Royal London Hospital School) described the experience of GC resistance in RA, a condition which again represents a major therapeutic challenge. In the course of a wide-ranging review, Dr Chikanza described studies on the influence of splice variants of the GR (GR β) which appear to be upregulated in severe RA cases. He went on to describe the role of lipocortin in mediating the effects of GCs and prolactin in counteracting these and discussed evidence indicating that perturbations of intracellular signalling may partially account for the incidence of steroid-resistant RA.

All in all the conference gave a very comprehensive analysis of the current understanding of steroid signalling including transcriptional, post-transcriptional and protein-protein effects. Perturbation of steroid signalling on any of these levels, may lead to a lack of steroid response. Further studies and an even better understanding of steroid signalling may in turn enable more treatment strategies for steroid resistant patients in the future. The discussion of these mechanisms in the context of both inflammatory and tumourigenic processes was particularly interesting for both the participants and those attending the conference, leading to a most productive and enjoyable meeting.

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Chair of Session