Chapter 5

Final Conclusion

The fact that neutrophilic inflammation is not controlled by glucocortico-steroids was acknowledged in the first chapter and remains an unmet need in the treatment of inflammatory conditions.

The research presented in this thesis was focused primarily on the effects of montelukast (ML) a selective cysteinyl leukotriene receptor-1 (CysLTR1) antagonist, on human neutrophils. CysLTR1-dependent and –independent activities were investigated.

The sensitising effects of the cysteinyl leukotrienes, C_4 and D_4, on human neutrophils and the antagonistic effects of ML demonstrated in Chapter two have not been documented previously. These pro-inflammatory interactions of CysLTs with neutrophils may play an important role in acute and chronic inflammatory conditions in both atopic and non-atopic individuals. This interaction between the CysLTs and human neutrophils may prove to be an important therapeutic target for control of neutrophilic inflammation in conditions where CysLTs have not been thought to play an important role. ML was also found to promote rapid clearance of Ca^{2+} from the cytosol of chemoattractant-activated neutrophils, which was associated with an increase in basal levels of cAMP and marked attenuation of the Ca^{2+}-dependent pro-inflammatory activities of the cells. At the same concentrations, ML was shown to possess non-specific PDE inhibitory activities, which appear to underpin the CysLTR1-independent inhibitory effects of this agent on neutrophil functions. When tested in combination, the anti-inflammatory effects of ML and formoterol were generally greater than those of the individual agents,

which may support the rationale of using both in combination with inhaled glucocortico-steroids in the treatment of asthma.

There are, however, several limitations to the current study, as well as potential future extensions. Firstly, the effects of ML on other types of inflammatory cells, including monocytes, macrophages and lymphocytes were not studied. This would be an important area of investigation given the fact that cAMP possesses broad spectrum anti-inflammatory activity encompassing cells of both the innate and adaptive immune systems (Moore & Willoughby, 1995; Serezani *et al*, 2008). Secondly, the effects of ML on other neutrophil-derived mediators of inflammation such as the chemokine IL-8 and the cytokine TNF were not studied. TNF levels have been reported to be decreased by ML in a rat model of experimental inflammation (Coskun *et al*. 2011), while it is well known that agents which elevate cAMP are potent inhibitors of the synthesis of TNF (Moore & Willoughby, 1995; Serezani *et al.*, 2008). Thirdly, the experiments described in the current study were only done *in vitro* and did not include an *ex vivo* component using blood samples of healthy volunteers or patients with bronchial asthma given montelukast at therapeutic doses.

Other aspects that require further investigation include:

- Investigation of the effects of montelukast on neutrophils from patients with chronic inflammatory disorders of the airways, especially bronchial asthma and chronic obstructive lung disease.

- Effects of montelukast on the pro-inflammatory activities of neutrophils activated with different activators such as IL-8, LTB_4 and TNF. The latter would be particularly interesting as it is not a Ca^{2+}-mobilizing activator and may not be responsive to montelukast.
- Effects of montelukast on the various purified isoforms of cAMP PDEs to establish their relative sensitivities to the agent
- Because of its anti-oxidative properties the effects of montelukast on neutrophil chemotaxis and phagocytosis are difficult to predict. Interference with NADPH oxidase activity could potentially predispose to bacterial infection and this aspect requires further investigation. However, there are no reports of increase susceptibility to bacterial or viral infections in patients receiving long-term therapy with montelukast.

In addition to these, future studies should include comparison of the anti-inflammatory effects of ML on neutrophils with those the other two commercially available CysLT1R antagonists, pranlukast and zafirlukast. Most importantly, however, assessment of the inflammatory potential of ML in genetically engineered mice with selective knockout of the gene encoding the CysLTR1 would be particularly revealing to probe the involvement of CysLTR1-independent anti-inflammatory activity in the therapeutic action of this agent.

Future clinical studies should also focus on the anti-inflammatory potential of other agents which combine CysLTR1 antagonism and PDE inhibitory activities. One

such agent is CR3465 which combines CysLTR1 antagonism with PDE3 and PDE4 inhibitory effects. A comparison was done with ML, demonstrating reversal of LTD4-induced bronchoconstriction, but not, however, comparing effects on PDE activity (Ferrari *et al*, 2004). Further comparative studies are necessary.

Likewise, the existence of other mechanisms of ML-mediated anti-inflammatory activity, distinct from those described in the current study cannot be discounted. For example, crosstalk between CysLTR1 and P2Y receptors has been described in a study in which the effects of the different CysLTR1 antagonists on human bronchial epithelial cells were investigated (Lau *et al*, 2011).

Importantly, the anti-inflammatory potential of ML in combination with other anti-inflammatory agents which target neutrophils by alternative mechanisms, especially the macrolides, also needs investigation. In this respect it is of interest that macrolides have the potential to modify the natural history of chronic inflammatory lung diseases including Post-transplant Bronchiolitis Obliterans Syndrome (BOS) (Friedlander *et al*, 2010). A pilot study done on patients with BOS already on a macrolide (azithromycin) to which ML was added, showed a significant arrest in FEV_1 decline relative to the group treated with the macrolide alone (Verleden *et al*, 2011). Currently, a multi-institutional phase II study of montelukast for the treatment of BOS is underway to which children older than 6 years of age and adults up to 80 years have been recruited. The outcome is awaited. (Martin, 2011)

Finally, the results of the current study have demonstrated that ML targets the pro-inflammatory activities of neutrophils by both CysLTR1-dependent and –independent mechanisms. However, the contribution of the latter to therapeutic activity, if any, remain to be conclusively established.