



Review

Equine recurrent airway obstruction and insect bite hypersensitivity: Understanding the diseases and uncovering possible new therapeutic approaches

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Accepted 3 October 2007

Abstract

Recurrent airway obstruction (RAO) and insect bite hypersensitivity (IBH) are allergic conditions that are commonly encountered in the horse. Whilst complete allergen avoidance is an effective management strategy for both diseases, this may not be achievable in all cases and treatment options are therefore required. The inflammatory response is the main therapeutic target for glucocorticoids given to horses with RAO and severe cases of IBH, whilst the bronchodilators used in RAO primarily target airway smooth muscle. Such drugs are effective in most but not all individuals and there may be unwanted adverse effects. This article will review how knowledge of drug action and the pathogenesis of RAO and IBH can be utilised to identify potential targets for novel therapeutic agents that, in the longer term, may be safer and/or more effective in managing the allergic horse.

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Keywords: Allergy; Horse; Insect bite hypersensitivity; Recurrent airway obstruction; RAO; Inflammation

Introduction

Equine allergic disease is most effectively managed by complete allergen avoidance but it is unlikely that this will be achievable in all cases. Therefore, safe and effective drugs are required for the treatment of common conditions such as recurrent airway obstruction (RAO), a term encompassing the chronic obstructive pulmonary condition seen on stabling or exposure of susceptible animals to summer pasture, and insect bite hypersensitivity (IBH), also known as ‘sweet itch’ or ‘summer eczema’.

The clinical signs of RAO, which can range from cough and exercise intolerance to severe respiratory distress, are caused by a hypersensitivity to inhaled organic dust. The presence of histamine and raised antigen-specific immunoglobulin (IgE) in the airways or serum of affected animals is

suggestive of a Type 1 hypersensitivity, despite the absence of an immediate response to allergen challenge (McGorum et al., 1993; Schmallenbach et al., 1998; Deaton et al., 2007; Künzle et al., 2007). The inflammatory response resulting from antigen exposure is largely responsible for the alterations in airway smooth muscle structure and function and is characterised by recruitment and activation of neutrophils and T lymphocytes, together with mucus hypersecretion (Robinson et al., 1996; Franchini et al., 1998, 2000; Lavoie et al., 2001; Robinson, 2001; Beadle et al., 2002; Giguère et al., 2002; Marr et al., 2002; Ainsworth et al., 2003, 2006; Léguillette, 2003; Cordeau et al., 2004; Desjardins et al., 2004; Brazil et al., 2005; Debrue et al., 2005; Horohov et al., 2005; Marinkovic et al., 2007).

IBH is an intensely pruritic, seasonally recurrent, hypersensitivity disorder to, as yet uncharacterised, allergens found largely in the saliva of biting midges (Foster and Cunningham, 1998a; Wilson et al., 2001; Althaus et al., 2004; Hellberg et al., 2006). As in RAO, histamine and IgE have been implicated in the pathogenesis of IBH,

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although the delayed response to intradermal allergen in some animals is suggestive of a Type 4, in addition to a Type 1, reaction (Riek, 1955; Anderson et al., 1991; Van der Haegen et al., 2001; Wagner et al., 2006a). The presence of antigen(s) in the skin of affected animals induces inflammatory cell infiltration, with early eosinophil accumulation followed by T cell recruitment. The T cell cytokine profile in IBH lesions has not been extensively studied although Marti et al. (2007) have recently described increased interleukin (IL)-4, IL-5 and IL-3 mRNA expression in acute lesions and increased chemokine (CCL11 and CCL2) mRNA expression has been reported in established lesions (Benarafa et al., 2002a). Increased expression of IL-1 β mRNA has also been detected although this may be due to mechanical trauma incurred when affected animals rub their skin to relieve the pruritus (Homey et al., 2006; Kolm et al., 2006). Such self-excoriation may also be, in part, responsible for the structural changes such as lichenification that are evident in chronically affected animals (Foster and Cunningham, 1998a).

Although clearly different conditions, affecting (primarily) disparate organ systems, equine RAO and IBH have in

common the development of an antigen-induced local inflammatory response, thought to be largely responsible for the subsequent short and long term structural and functional changes in the lungs or skin, in which lymphocytes appear to play a key role and which involves recruitment and activation of circulating granulocytes. This being the case, it is not surprising that allergen avoidance or administration of drugs with a broad profile of anti-inflammatory actions such as glucocorticoids are found to be effective approaches in managing both conditions. As current understanding of the molecular events regulating key inflammatory processes in the horse increases, the extent to which compounds with more selective anti-inflammatory properties will be effective in RAO and/or IBH will become increasingly apparent.

This review will first summarise the main drug classes currently used for these conditions and the ways in which they might be improved and then discuss some potential new therapeutic targets that studies of disease pathogenesis in the horse have suggested could be of interest. A summary of potential new therapeutic approaches to the management of RAO and IBH is given in Tables 1 and 2.

Table 1
Summary of potential new therapeutic approaches to the management of RAO

Potential therapy	Comments
Low dose theophylline	Several weeks treatment likely to be required before any anti-inflammatory effect is observed
Lipoxins	The role of anti-inflammatory lipoxins in RAO is unknown
Cytokine/chemokine inhibitors (IL-4, IL-13, TNF α , IFN γ , CXCL8)	The role of individual cytokines and chemokines in RAO is not yet fully understood Blockade of the effects of a single cytokine/chemokine in established disease has not yet been shown to be of significant benefit in similar complex clinical conditions in man
Transcription factor inhibitors (NF κ B)	Adverse effects may limit use
<i>Inhibitors of intracellular enzymes</i>	
PDE (PDE4/7)	PDE4 inhibition has not been shown to be of benefit in RAO (Rickards et al., 2003; Lavoie et al., 2006) and PDE7 has not yet been identified in equine cells
p38 MAPK	p38 MAPK inhibition has been shown to be of some benefit but only if administered prior to antigen exposure (Lavoie et al., 2004) May be difficult to achieve an acceptable therapeutic window and adverse effects may limit use
Anti-IgE therapy	The involvement of IgE has not been conclusively demonstrated

Table 2
Summary of potential new therapeutic approaches to the management of IBH

Potential therapy	Comments
H ₄ receptor antagonists	Unlikely to succeed if used as sole therapy H ₄ receptor involvement in pruritus has not been established in the horse There are a large number of potential pruritogens in addition to histamine
Leukotriene inhibitors	The role of arachidonic acid metabolites in IBH is unknown
Cytokine/chemokine inhibitors (IL-4, IL-5, IL-13, TNF α , IFN γ , CCL11)	The role of individual cytokines and chemokines in IBH is not yet fully understood Blockade of the effects of a single cytokine/chemokine in established disease has not yet been shown to be of significant benefit in similar complex clinical conditions in man
<i>Inhibitors of intracellular enzymes</i>	
Calcineurin	Cyclosporine is effective in canine atopic dermatitis but drug pharmacokinetics and toxicity following systemic administration in horses are unknown Treatment will be expensive
PDE (PDE4, PDE4/7)	The role of PDE4 in IBH has not been investigated and PDE7 has not yet been identified in equine cells
Mycobacterium based vaccine	Trials ongoing with reports of improvement in some individuals
Anti-IgE therapy	Some success in the treatment of human atopic eczema

Current therapeutic options for RAO and IBH

The inflammatory events taking place in the lungs or skin of horses with RAO or IBH are the broad therapeutic target for glucocorticoids which are the drugs of choice for maintenance therapy when management strategies are insufficient. However, whilst they may be regarded as ‘the drugs to beat’, in view of the pleiotropic effects of the glucocorticoid-receptor complex, their use can be associated with unwanted effects (Barnes, 2006a). In the horse there is a particular concern about the onset or exacerbation of laminitis although the association has yet to be proved (Bailey and Elliott, 2007). Prophylactic administration of sodium cromoglycate is another approach to controlling the inflammatory response in RAO but the drug is not used commonly in equine or human allergic airway disease (Thomson and McPherson, 1981; Larsson et al., 2001; Crompton, 2006). In IBH, histamine (H)₁ receptor antagonists have been used therapeutically but with variable success (Foster and Cunningham, 1998a).

Although bronchoconstriction in RAO is largely due to the inflammatory events taking place in the airways, bronchodilator therapy is still an important part of disease management. The most commonly used bronchodilators target beta (β)₂ or muscarinic receptors on airway smooth muscle, although β ₂ agonists have some, additional, anti-inflammatory properties (Broadley, 2006; Laan et al., 2006a; van den Hoven et al., 2006). Clenbuterol, the only β ₂ agonist authorised for use in horses, is not effective in all patients (Léguillette, 2003) which may be due, in part, to the reduction in β ₂ receptor density associated with administration of this class of drug. Receptor numbers and the efficiency of receptor coupling to adenylate cyclase have also been reported to be reduced in RAO horses (Abraham et al., 2002, 2006). The muscarinic receptor antagonist atropine is an effective bronchodilator, but, as it is non-selective in its action on receptor sub-types, systemic use may lead to reduced gastrointestinal tract motility and other unwanted effects (Léguillette, 2003; Gosens et al., 2006). These may be limited by using inhaled ipratropium bromide, another muscarinic antagonist (Duvivier et al., 1997).

Theophylline is used clinically as a bronchodilator in man and in other domestic species but not routinely in horses due to the tachycardia and excitement that occur within the therapeutic range (McKiernan et al., 1990). The mechanism by which theophylline produces its bronchodilator effect is not fully understood but adenosine receptors and phosphodiesterase (PDE) in airway smooth muscle have been suggested as targets for drug action. It is additionally recognised that theophylline can exert anti-inflammatory effects at lower doses than required for bronchodilation (Barnes, 2002; see later).

Improving existing therapeutic agents for equine RAO and IBH

From the discussion above, it is clear that there are suitable therapeutic options for RAO and IBH but there are

some limitations to their use. Advances in knowledge of their pharmacodynamics and pharmacokinetics have led to suggestions about how existing drugs could be improved.

Glucocorticoids

At a molecular level the anti-inflammatory effects of glucocorticoids are mediated primarily by trans-repression of gene transcription, leading to a reduction in the formation of a range of inflammatory proteins, including many cytokines and chemokines, adhesion molecules, enzymes and receptors. Through trans-activation there is also increased expression of anti-inflammatory peptides and β ₂ adrenoreceptors are also up-regulated (Barnes, 2006a). Many of the unwanted effects are due to trans-activation and hence steroidal and non-steroidal glucocorticoid receptor agonists have been developed that show selectivity for trans-repression and should, therefore, have an improved safety profile (Schäcke et al., 2002, 2004). Species differences in the trans-activation response have been reported which may need to be taken into account if drugs with selective trans-repressor action are to be identified for use in the horse (Tanigawa et al., 2002).

Methylxanthines

The inhibitory effects of theophylline on inflammatory cell accumulation and activation in human asthmatics referred to earlier occur at lower plasma concentrations than are required for bronchodilation in horses (Button et al., 1985; Sullivan et al., 1994). However, several weeks of oral therapy are necessary before the anti-inflammatory effects become evident (Culpitt et al., 2002; Kobayashi et al., 2004). Thus, although it is of interest that Cesarini et al. (2006) reported the lack of any beneficial effect of low dose oral theophylline in horses with RAO, the animals were only treated for 7 days and, as no indices of inflammation were reported, the dose or duration of treatment may have been insufficient.

The mechanism by which theophylline exerts its anti-inflammatory action is not yet fully understood but enhanced activity of histone deacetylase (HDAC) in vitro and ex vivo following 4 weeks treatment with low dose theophylline has been demonstrated (Ito et al., 2002; Cosio et al., 2004). HDACs are co-repressor proteins that deacetylate core histones, thereby suppressing inflammatory gene expression. If a similar action can be demonstrated in the horse, and inhibitory effects on leucocyte accumulation and activation observed, it may prove possible to use theophylline safely and effectively in RAO.

Histamine receptor antagonists

More than 50 years ago, Riek (1955) provided evidence that histamine was involved in the pathogenesis of IBH. H₁ receptor antagonists may act in part by reducing pruritus

and, in part, by inhibiting histamine-induced swelling and leucocyte recruitment (Molet et al., 1997; Foster and Cunningham, 1998a,b; Foster et al., 1998; Bailey and Cunningham, 2001a). Recently, studies using knock out mice have suggested that histamine induces pruritus largely through activation of H_4 receptors and, as H_4 receptor antagonists also have anti-inflammatory properties (Dunford et al., 2007), this may be a more appropriate therapeutic target in IBH than the H_1 receptor. Moreover, given the number of pruritogens that have now been identified (Paus et al., 2006), it seems likely that other innovative approaches to reducing pruritus will be developed.

Although histamine has been recovered from the lungs of symptomatic RAO horses, targeting H_1 receptors has not proved an effective strategy in this condition (McGorum et al., 1993; Robinson, 2001). Some benefit has been reported following administration of second generation H_1 receptor antagonists to human patients with mild to moderate asthma, although higher than conventional doses were required (Nelson, 2003). If second or third generation drugs with favourable pharmacokinetics can be identified for use in the horse (Olsén et al., 2006), it could be worth evaluating their use as sole agents or as part of a combination therapy in RAO.

Muscarinic antagonists

Tiotropium bromide is a muscarinic receptor antagonist that shows 'pharmacokinetic selectivity', in that it dissociates slowly from M3 and M1 receptor sub-types (Disse et al., 1999). It is an effective bronchodilator in obstructive lung disease in man when given once daily by inhalation and reduces the decline in lung function changes in patients with chronic obstructive pulmonary disease (Gosens et al., 2006). If the favourable pharmacokinetic and pharmacodynamic properties demonstrated in man are observed in the RAO horse, this drug may prove to be superior to ipratropium bromide as an inhaled therapy.

Potential novel therapeutic targets for RAO and IBH

Studies of allergic inflammation and diseases such as asthma and atopic eczema in man have yielded a plethora of potential therapeutic targets, some or all of which may be applicable to the horse (Adcock et al., 2006; Barnes, 2006b; Boswell-Smith et al., 2006; Homey et al., 2006; Paus et al., 2006; Pua and Barnetson, 2006; Yamagata and Ichinose, 2006; Baumer et al., 2007). IBH shares features with atopic eczema (Foster and Cunningham, 1998a; Boguniewicz and Leung, 2006) and RAO is sometimes referred to as 'equine asthma', although the disease also has similarities to hypersensitivity pneumonitis and a contribution to lung inflammation and injury from lipopolysaccharide and inhaled particles must also be acknowledged (Pirie et al., 2003a,b; Ghio et al., 2006; Ismail et al., 2006).

Although differences between the pathogenesis of IBH and RAO and analogous conditions in other species exist

and treatment protocols and drug pharmacodynamics and pharmacokinetics may differ in the horse, which could influence the outcome of any novel therapeutic strategy, it nevertheless seems reasonable to suggest that much of the knowledge gained from animal models of human disease and in patient studies will be applicable to the equine conditions. This review will focus on those targets that studies of disease pathogenesis in the allergic horse have suggested could be of potential interest.

Arachidonic acid metabolites

Despite leukotrienes (LTs) having been implicated in bronchoconstriction and airway neutrophilia in RAO, neither antagonism of the LTD₄ receptor, nor 5-lipoxygenase inhibition, were found to be of benefit although the study on montelukast is inconclusive as the authors did not show inhibition of LTD₄ at the dose used and the pharmacokinetic data suggested poor oral bioavailability (Marr et al., 1998a,b,c; Lavoie et al., 2002; Kolm et al., 2003; Lindberg et al., 2004). Taken together, these findings do not encourage further investigation of leukotriene inhibition in RAO although it may be worth noting that in some European countries LTD₄ antagonists are approved as an add-on therapy in asthma when glucocorticoids are insufficient or for prophylactic use in exercise-induced bronchoconstriction in asthmatics (Dahlen, 2006). The role of arachidonic acid metabolites has not been studied in IBH and there is some evidence for therapeutic improvement in human atopic eczema following administration of LTD₄ receptor antagonists (Pua and Barnetson, 2006). Whether these, or 5-lipoxygenase inhibitors, would prove useful in treating allergic skin disease in the horse remains to be established.

Whilst inhibition of pro-inflammatory eicosanoids may not be an effective therapeutic strategy, the anti-inflammatory lipoxins may represent a potential target. Lipoxins are short lived tri-hydroxy eicosanoids produced by transcellular synthesis involving 5-lipoxygenase (LOX) and, primarily, 15-LOX. Lipoxins, which activate formyl peptide receptor like-1 (FPRL1) receptors, reducing inflammatory and epithelial cell activation (Parkinson, 2006), have been detected in bronchoalveolar lavage (BAL) from patients with inflammatory lung disease and production by leucocytes from asthmatic subjects is reported to increase in mild disease. However, in severe cases where there is persistent inflammation, a significant reduction has been identified (Bonnans et al., 2004; Levy et al., 2007). Lipoxins have not been studied in RAO but the report of raised 15-HETE in symptomatic horses is compatible with increased 15-LOX activity and, potentially, lipoxin formation (Gray et al., 1992). It would be of interest to determine whether there is an imbalance between the production of pro- and anti-inflammatory eicosanoids in RAO that is associated with disease severity, as has been observed in the severe asthmatic and in cows with persistent mastitis (Boutet

et al., 2003), and if drugs that mimic the actions of lipoxins might be beneficial therapeutically.

Cytokines and chemokines

Cytokines and chemokines are key mediators in orchestrating the immune response and, as such, represent a rational therapeutic target in the allergic patient (Homey et al., 2006; O'Byrne, 2006; Pua and Barnetson, 2006; Woodfolk, 2006; Yamagata and Ichinose, 2006). There have been a number of studies describing increased mRNA expression for a range of cytokines and chemokines in bronchoalveolar lavage (BAL) cells, epithelium or lymphocytes from RAO horses and in the lesional skin of IBH horses (Franchini et al., 1998; Lavoie et al., 2001; Beadle et al., 2002; Benarafa et al., 2002a; Giguère et al., 2002; Ainsworth et al., 2003, 2006; Cordeau et al., 2004; Desjardins et al., 2004; Debrue et al., 2005; Horohov et al., 2005; Kleiber et al., 2005; Kolm et al., 2006; Marti et al., 2007).

Determining whether or not such changes are reflected by an increase in expressed protein is currently hampered by the lack of suitable reagents and, to date, only an increase in CXCL8 (IL-8) in BAL fluid from RAO horses has been reported when compared to healthy controls (Franchini et al., 2000; Giguère et al., 2002). Nevertheless, the increase in mRNA expression, together with the documented properties of the chemokines and cytokines, provides a sufficient basis for suggesting that the effects of selective blockade warrant investigation in the allergic horse.

IL-4

The recent report linking the region of chromosome 13 containing the IL-4 receptor α gene to RAO, together with the multiple immunoregulatory effects of the cytokine in allergic disease and the increased mRNA expression reported both in the lesional skin of horses with IBH and, in some studies, in BAL cells from symptomatic RAO horses, suggests that inhibiting IL-4 may be worth investigating in these conditions (Lavoie et al., 2001; Beadle et al., 2002; Giguère et al., 2002; Jackson et al., 2004; Horohov et al., 2005; Homey et al., 2006; O'Byrne, 2006; Pua and Barnetson, 2006; Wagner et al., 2006b; Woodfolk, 2006; Yamagata and Ichinose, 2006; Jost et al., 2007; Marti et al., 2007). However, studies with monoclonal antibodies and an IL-4 soluble receptor in human asthma have yielded disappointing results, with only modest beneficial effects being reported. Whether early intervention, rather than in established disease, might be of greater benefit remains to be established (O'Byrne, 2006).

IL-5

Eosinophils play a role in the pathogenesis of human asthma but, although blocking IL-5 reduced eosinophil accumulation and blood eosinophilia, no improvement in

airway function was observed suggesting that targeting IL-5 is not a useful approach in this condition (O'Byrne, 2006; Yamagata and Ichinose, 2006). The cytokine is of less interest as a therapeutic target in RAO because of the limited involvement of eosinophils (Robinson et al., 1996; Robinson, 2001; Léguillette, 2003) although increased expression of IL-5 mRNA in BAL cells from symptomatic RAO horses has been reported in some studies (Lavoie et al., 2001; Giguère et al., 2002; Cordeau et al., 2004) and IL-5 receptors may be present on neutrophils from these animals (Dewachi et al., 2005). In contrast to RAO, early eosinophil recruitment to the skin is a feature of IBH (Foster and Cunningham, 1998a). Equine eosinophil adherence in response to IL-5 is increased to a significantly greater extent in ponies with IBH and IL-5 mRNA is increased in acute IBH lesions (Bailey and Cunningham, 2001b; Cunningham et al., 2003; Marti et al., 2007). However, as in asthma, anti-IL-5 therapy has not proved to be of significant benefit in human atopic eczema (Oldhoff et al., 2005). Thus, at present, IL-5 does not appear to be an attractive target for blockade in equine allergic disease.

Tumour necrosis factor (TNF) α

TNF α mRNA is increased in BAL cells from symptomatic RAO horses, although no change in the level of expressed protein was detected (Giguère et al., 2002; Laan et al., 2006b). Preliminary reports on blockade of TNF α in asthma and atopic eczema appear promising and this approach could prove to be beneficial in equine RAO or IBH (Jacobi et al., 2005; Erin et al., 2006; O'Byrne, 2006; Yamagata and Ichinose, 2006). Inhibition of TNF α converting enzyme (TACE), which cleaves the cytokine from the cell surface, is an alternative mechanism by which the actions of TNF α can be modulated and a TACE inhibitor has been shown to modulate cytokine release by equine macrophages (Wijnker et al., 2004; Lombart et al., 2007).

Interferon (IFN) γ

Subcutaneous administration of IFN γ is associated with clinical improvement in both human and canine patients with atopic dermatitis (Iwasaki and Hasegawa, 2006; Pua and Barnetson, 2006). Whether IFN γ is also a rational target in equine IBH remains to be established in light of the recent finding that expression of IFN γ mRNA is not altered in the skin of IBH horses (Marti et al., 2007). IFN γ mRNA expression has been reported to be decreased in BAL cells from RAO horses in some studies (Lavoie et al., 2001; Cordeau et al., 2004) and increased in others (Beadle et al., 2002; Giguère et al., 2002; Ainsworth et al., 2003; Horohov et al., 2005), something which may be explained by differences in sampling times and the clinical stage of the study animals (Horohov et al., 2005). Thus, as in human asthma and equine IBH, it is not yet clear if IFN γ administration would be a useful approach in RAO (Yamagata and Ichinose, 2006).

IL-13

IL-13 is thought to play a key role in the pathogenesis of allergic disease (Wills-Karp, 2004; Homey et al., 2006; Yamagata and Ichinose, 2006) and administration of an IL-13 monoclonal antibody has been found to reduce the response to antigen challenge in primates (Bree et al., 2007). With evidence of increased IL-13 mRNA expression in BAL cells from RAO horses (Horohov et al., 2005) and in acute IBH lesions (Marti et al., 2007), blocking the effects of this cytokine may prove beneficial, although a recent study in mice suggests that dual IL-4/IL-13 inhibition could be more effective (Perkins et al., 2006).

CXCL8 (IL-8)

Increased CXCL8 and mRNA expression have been reported in epithelial cells and BAL fluid from symptomatic RAO horses (Franchini et al., 2000; Giguère et al., 2002; Ainsworth et al., 2003, 2006) and this neutrophil activating chemokine has been implicated in causing airway neutrophilia. There has been some interest in examining the impact of CXCL8 inhibition in chronic obstructive pulmonary disease in man but the beneficial effect obtained was very limited (Chung, 2006). As neutrophil accumulation can be dissociated from lung function changes in RAO (Fairbairn et al., 1993), targeting up-stream cytokines responsible for the release of CXCL8 may be a more effective strategy (Debrue et al., 2005; Ainsworth et al., 2006).

CCL11 (eotaxin) and CCR3

Targeting CCR3, the receptor for CCL11, which is a potent eosinophil and T cell chemoattractant (Benarafa et al., 2002b; Weston et al., 2006a), is an approach that is of potential interest in the treatment of allergic skin disease in man, although there are, as yet, no published studies describing the effects of CCR3 antagonists in clinical patients (De Lucca, 2006). CCL11 mRNA is increased in equine fibroblasts stimulated with IL-4 and in the lesional skin of IBH horses when eosinophils are present (Benarafa et al., 2000, 2002a). Studies of the chemokine and its receptor will be facilitated by the partial sequencing and consequent design of primers against equine CCR3 (Weston et al., 2006b).

Mycobacterium spp.

It is recognised that bacterial antigens modulate immune cell function, promoting Th1 cytokine-dependent responses and suppressing IgE synthesis (Renz and Herz, 2002; Camporota et al., 2003). An ongoing study of a mycobacterium based vaccine in IBH is reported to be showing promising results (Stanford, 2007) and investigating such a vaccine in RAO could be of interest as a single intradermal injection of *Mycobacterium vaccae* was reported to

reduce the late phase response following bronchial provocation in human asthmatics (Camporota et al., 2003).

Transcription factors

Activation of transcription factors is a necessary step in the synthesis of inflammatory proteins including cytokines and chemokines and inhibiting those that are activated or over-expressed in disease represents a potential target, although adverse effects may limit the use of this approach as an anti-inflammatory therapy (Adcock et al., 2006; Barnes, 2006b). Increased expression of a p65 homodimeric form of nuclear factor- κ B (NF κ B) has been reported in BAL and epithelial cells from symptomatic RAO horses (Bureau et al., 2000; Sandersen et al., 2001). Low molecular weight inhibitors of IKK2, the isoenzyme that is important for activation of NF κ B by inflammatory stimuli, have been produced and shown to reduce cytokine and chemokine synthesis by human pulmonary epithelial cells (Newton et al., 2007). Such compounds have yet to be tested in allergic patients.

Intracellular enzymes

Calcineurin

The nuclear factor of activated T cells (NF-AT) regulates mRNA transcription of cytokines, and inhibitors of the phosphatase calcineurin, e.g. cyclosporine, will prevent dephosphorylation of the cytoplasmic component of NF-AT, thereby reducing cytokine production. In addition, cyclosporine reduces mediator release by mast cells and basophils (Pua and Barnetson, 2006).

Cyclosporine is authorised for use in canine atopic dermatitis and could be of benefit in severe cases of IBH, although expense is likely to limit its use in the horse. Moreover, whilst topical cyclosporine is used in equine ophthalmology, drug toxicity would need to be evaluated in this species if systemic administration was envisaged (Gilger et al., 2005; Steffan et al., 2006).

Kinases

P38 MAPK is a family of four serine threonine protein kinase isoenzymes involved in regulating many cellular responses and selective inhibition of p38 MAPK activation has been shown to be effective in animal models of human asthma and COPD (Adcock et al., 2006; Barnes, 2006b). Lavoie et al. (2004) have reported some beneficial effects in horses with RAO after administration of a p38 MAPK inhibitor prior to antigen exposure, with clinical signs being reduced in severity and delayed in onset. Moreover, there was a reduction in airway neutrophilia to which inhibition of LPS-induced p38 MAPK activation in leucocytes may have contributed (Pirie et al., 2003a,b; Brooks et al., 2007). Inhibition of intracellular signalling molecules involved in transducing signals from multiple extracellular

inflammatory stimuli is an attractive concept. However, given that kinases, such as p38 MAPK, play an important regulatory role in so many different cellular events, compounds that are highly selective in action are likely to be required in order to achieve an acceptable therapeutic index and administration by inhalation may also be necessary to limit unwanted effects.

Phosphodiesterase (PDE)

PDE catalyses the breakdown of intracellular cAMP and identification of a family of at least 11 isoenzymes with over 50 isoforms that are differentially expressed in almost all mammalian tissues, again offers the potential for selective inhibition (Boswell-Smith et al., 2006). As in other species, PDE4 is the main isoenzyme found in equine lymphocytes and neutrophils (Rickards et al., 2000, 2004a).

The activity of PDE isoenzymes, including PDE4, was similar in cells from RAO and healthy horses, although some differences in the control of lymphocyte PDE3 and PDE5 activity were identified (Rickards et al., 2000, 2004b), and inhibition of PDE4 was not associated with any improvement in airway function or inflammation in symptomatic animals, suggesting that targeting PDE4 may not be worthwhile (Rickards et al., 2003; Lavoie et al., 2006). PDE4 inhibition has been associated with anti-inflammatory effects in animal models of allergic skin inflammation where suppression of cytokine formation has been demonstrated, and in early clinical trials of atopic eczema in man (Baumer et al., 2007). In addition, Ferrer et al. (1999) have reported improvement equivalent to that observed with prednisolone in canine atopic dermatitis. Thus, PDE4 inhibitors may be worthy of evaluation in IBH. Moreover, PDE7 is also present in inflammatory cells and combined inhibition of PDE4 and PDE7 may be a more effective strategy (Boswell-Smith et al., 2006).

IgE

Wagner et al. (2006a) have demonstrated a role for IgE in mediating Type I hypersensitivity reactions in the horse. There is also evidence for IgE involvement in the pathogenesis of IBH and, perhaps less convincingly, in RAO (Schmallenbach et al., 1998; Van der Haegen et al., 2001; Wilson et al., 2006; Künzle et al., 2007). Anti-IgE therapy, which has been attempted with some success in human asthma and atopic eczema (Chang et al., 2007) may warrant investigation in the allergic horse.

Conclusions

Although studies of the pathogenesis of RAO and IBH have identified a variety of potential therapeutic targets worthy of evaluation, it is clear that there is still much to be learnt about the mediators and mechanisms underlying the inflammatory response and the associated structural and functional changes in these diseases. Current evidence

suggests that, in many cases, blocking individual mediators may only lead to limited improvement, whereas achieving a broader anti-inflammatory action by, for example, targeting transcription factors or intracellular signalling molecules, could ultimately be unsuccessful if an acceptable therapeutic window cannot be achieved. Whilst such novel approaches are being investigated, optimising the use of existing groups of drugs through a better understanding of their actions at the cellular and molecular level could be of benefit in treating RAO and IBH.

Acknowledgements

We would like to acknowledge all those whose work is referred to, in particular those colleagues with whom we have had the pleasure of working, and the financial support provided by the Horse Trust and the Horserace Betting Levy Board, which has been invaluable in pursuing our investigations of IBH and RAO.

References

- Abraham, G., Brodde, O.E., Ungemach, F.R., 2002. Regulation of equine lymphocyte beta-adrenoceptors under the influence of clenbuterol and dexamethasone. *Equine Veterinary Journal* 34, 587–593.
- Abraham, G., Kottke, C., Dhein, S., Ungemach, F.R., 2006. Agonist-independent alteration in β -adrenoceptor-G-protein-adenylate cyclase system in an equine model of recurrent airway obstruction. *Pulmonary Pharmacology and Therapeutics* 19, 218–229.
- Adcock, I.M., Chung, K.F., Caramori, G., Ito, K., 2006. Kinase inhibitors and airway inflammation. *European Journal of Pharmacology* 533, 118–132.
- Ainsworth, D.M., Grünig, G., Matychak, M-B., Young, J., Wagner, B., Erb, H.N., Antczak, D.F., 2003. Recurrent airway obstruction (RAO) in horses is characterized by IFN- γ and IL-8 production in bronchoalveolar lavage cells. *Veterinary Immunology and Immunopathology* 96, 83–91.
- Ainsworth, D.M., Wagner, B., Franchini, M., Grünig, G., Erb, H.N., Tan, J-Y., 2006. Time-dependent alterations in gene expression of interleukin-8 in the bronchial epithelium of horses with recurrent airway obstruction. *American Journal of Veterinary Research* 67, 669–677.
- Althaus, H., Müller, N., Busato, A., Mellor, P.S., Torsteinsdottir, S., Marti, E., 2004. Cloning and sequencing of a cDNA expressing a ribosomal P0 peptide from *Culicoides nubeculosus* (Diptera). *Veterinary Immunology and Immunopathology* 99, 99–111.
- Anderson, G.S., Belton, P., Kleider, N., 1991. *Culicoides obsoletus* (Diptera: Ceratopogonidae) as a causal agent of *Culicoides* hypersensitivity (sweet itch) in British Columbia. *Journal of Medical Entomology* 28, 685–693.
- Bailey, S.R., Cunningham, F.M., 2001a. Inflammatory mediators induce endothelium-dependent adherence of equine eosinophils to cultured endothelial cells. *Journal of Veterinary Pharmacology and Therapeutics* 24, 209–214.
- Bailey, S.R., Cunningham, F.M., 2001b. Adherence of eosinophils from allergic and normal ponies to cultured equine endothelial cells. *Inflammation Research* 50, 32–38.
- Bailey, S.R., Elliott, J., 2007. The corticosteroid laminitis story: 2. Science of if, when and how. *Equine Veterinary Journal* 39, 7–11.
- Barnes, P.J., 2002. Theophylline. New perspectives for an old drug. *American Journal of Respiratory and Critical Care Medicine* 167, 813–818.
- Barnes, P.J., 2006a. Corticosteroids: The drugs to beat. *European Journal of Pharmacology* 533, 2–14.

- Barnes, P.J., 2006b. Novel signal transduction modulators for the treatment of airway diseases. *Pharmacology and Therapeutics* 109, 238–245.
- Baumer, W., Hoppmann, J., Rundfeldt, C., Kietzmann, M., 2007. Highly selective phosphodiesterase 4 inhibitors for the treatment of allergic skin diseases and psoriasis. *Inflammation Allergy Drug Targets* 6, 17–26.
- Beadle, R.E., Horohov, D.W., Gaunt, S.D., 2002. Interleukin-4 and interferon-gamma gene expression in summer pasture-associated obstructive pulmonary disease affected horses. *Equine Veterinary Journal* 34, 389–394.
- Benarafa, C., Cunningham, F.M., Hamblin, A.S., Horohov, D., Collins, M.E., 2000. Cloning of equine chemokines eotaxin, monocyte chemoattractant protein (MCP)-1, MCP-2 and MCP-4, mRNA expression in tissues and induction by IL-4 in dermal fibroblasts. *Veterinary Immunology and Immunopathology* 76, 283–298.
- Benarafa, C., Collins, M.E., Hamblin, A.S., Cunningham, F.M., 2002a. A role for the chemokine eotaxin in the pathogenesis of equine sweet itch. *Veterinary Record* 151, 691–693.
- Benarafa, C., Collins, M.E., Hamblin, A.S., Sabroe, I., Cunningham, F.M., 2002b. Characterisation of the biological activity of recombinant equine eotaxin in vitro. *Cytokine* 19, 27–30.
- Boguniewicz, M., Leung, D.Y.M., 2006. 10. Atopic dermatitis. *Journal of Allergy and Clinical Immunology* 117, S475–S480.
- Bonnans, C., Chanez, P., Chavis, C., 2004. Lipoxins in asthma: potential therapeutic mediators on bronchial inflammation? *Allergy* 59, 1027–1041.
- Boswell-Smith, V., Spina, D., Page, C.P., 2006. Phosphodiesterase inhibitors. *British Journal of Pharmacology* 147, S252–S257.
- Boutet, P., Bureau, F., Degand, G., Lekeuk, P., 2003. Imbalance between lipoxin A₄ and leukotriene B₄ in chronic mastitis-affected cows. *Journal of Dairy Science* 86, 3430–3439.
- Brazil, T.J., Dagleish, M.P., McGorum, B.C., Dixon, P.M., Haslett, C., Chilvers, E.R., 2005. Kinetics of pulmonary neutrophil recruitment and clearance in a natural and spontaneously resolving model of airway inflammation. *Clinical and Experimental Allergy* 35, 854–865.
- Bree, A., Schlerman, F.J., Wadanoli, M., Tchistiakova, L., Marquette, K., Tan, X.-Y., Jacobson, B.A., Widom, A., Cook, T.A., Wood, N., Vunnum, S., Krykbaev, R., Xu, X., Donaldson, D.D., Goldman, S.J., Sypek, J., Kasaian, M.T., 2007. IL-13 blockade reduces lung inflammation after *Ascaris suum* challenge in cynomolgus monkeys. *Journal of Allergy and Clinical Immunology* 119, 1251–1257.
- Broadley, K.J., 2006. β -Adrenoceptor responses of the airways: For better or worse? *European Journal of Pharmacology* 533, 15–27.
- Brooks, A.C., Menzies-Gow, N.J., Wheeler-Jones, C., Bailey, S.R., Cunningham, F.M., Elliott, J., 2007. Endotoxin-induced activation of equine platelets: evidence for direct activation of p38 MAPK pathways and vasoactive mediator production. *Inflammation Research* 56, 154–161.
- Bureau, F., Bonizzi, G., Kirschvink, N., Delhalle, S., Desmecht, D., Merville, M.-P., Bours, V., Lekeux, P., 2000. Correlation between nuclear factor- κ B activity in bronchial brushing samples and lung dysfunction in an animal model of asthma. *American Journal of Respiratory and Critical Care Medicine* 161, 1314–1321.
- Button, C., Errecalde, J.O., Mulders, M.S., 1985. Loading and maintenance dosage regimens for theophylline in horses. *Journal of Veterinary Pharmacology and Therapeutics* 8, 328–330.
- Camporota, L., Corkhill, A., Long, H., Lordan, J., Stanciu, L., Tuckwell, N., Cross, A., Stanford, J.L., Rook, G.A.W., Holgate, S.T., Djukanovic, R., 2003. The effects of *Mycobacterium vaccae* on allergen-induced airway responses in atopic asthma. *European Respiratory Journal* 21, 287–293.
- Cesarini, C., Hamilton, E., Picandet, V., Lavoie, J.-P., 2006. Theophylline does not potentiate the effects of a low dose of dexamethasone in horses with recurrent airway obstruction. *Equine Veterinary Journal* 38, 570–573.
- Chang, T.W., Wu, P.C., Hsu, C.L., Hung, A.F., 2007. Anti-IgE antibodies for the treatment of IgE-mediated allergic diseases. *Advances in Immunology* 93, 63–119.
- Chung, K.F., 2006. Cytokines as targets in chronic obstructive pulmonary disease. *Current Drug Targets* 7, 675–681.
- Cordeau, M.-E., Joubert, P., Dewachi, O., Hamid, Q., Lavoie, J.-P., 2004. IL-4, IL-5 and IFN- γ mRNA expression in pulmonary lymphocytes in equine heaves. *Veterinary Immunology and Immunopathology* 97, 87–96.
- Cosio, B.G., Tsaprouni, L., Ito, K., Jazrawi, E., Adcock, I.M., Barnes, P.J., 2004. Theophylline restores histone deacetylase activity and steroid responses in COPD macrophages. *Journal of Experimental Medicine* 200, 689–695.
- Crompton, G., 2006. A brief history of inhaled asthma therapy over the last fifty years. *Primary Care Respiratory Journal* 15, 326–331.
- Culpitt, S.V., de Matos, C., Russell, R.E., Donnelly, L.E., Rogers, D.F., Barnes, P.J., 2002. Effect of theophylline on induced sputum inflammatory indices and neutrophil chemotaxis in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* 165, 1371–1376.
- Cunningham, F.M., Vandergriff, E., Bailey, S.R., Sepulveda, M.F., Goode, N.T., Horohov, D.W., 2003. Cloning, expression and biological activity of equine interleukin (IL)-5. *Veterinary Immunology and Immunopathology* 95, 63–72.
- Dahlen, S.E., 2006. Treatment of asthma with antileukotrienes: first or last resort therapy? *European Journal of Pharmacology* 533, 40–56.
- De Lucca, G.V., 2006. Recent developments in CCR3 antagonists. *Current Opinion in Drug Discovery and Development* 9, 516–524.
- Deaton, C.M., Deaton, L., Jose-Cunilleras, E., Vincent, T.L., Baird, A.W., Dacre, K., Marlin, D.J., 2007. Early airway obstruction in response to organic dust in the horse. *Journal of Applied Physiology* 102, 1071–1077.
- Debrue, M., Hamilton, E., Joubert, P., Lajoie-Kadoch, S., Lavoie, J.-P., 2005. Chronic exacerbation of equine heaves is associated with an increased expression of interleukin-17 mRNA in bronchoalveolar lavage cells. *Veterinary Immunology and Immunopathology* 105, 25–31.
- Desjardins, I., Theoret, C., Joubert, P., Wagner, B., Lavoie, J.-P., 2004. Comparison of TGF- β 1 concentrations in bronchoalveolar fluid of horses affected with heaves and of normal controls. *Veterinary Immunology and Immunopathology* 101, 133–141.
- Dewachi, O., Joubert, P., Hamid, Q., Lavoie, J.-P., 2005. Expression of interleukin (IL)-5 and IL-9 receptors on neutrophils of horses with heaves. *Veterinary Immunology and Immunopathology* 109, 31–36.
- Disse, B., Speck, G.A., Rominger, K.L., Witek Jr., T.J., Hammer, R., 1999. Tiotropium (Spiriva): mechanistical considerations and clinical profile in obstructive lung disease. *Life Sciences* 64, 457–464.
- Dunford, P.J., Williams, K.N., Desai, P.J., Karlsson, L., McQueen, D., Thurmond, R.L., 2007. Histamine H₄ receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *Journal of Allergy and Clinical Immunology* 119, 176–183.
- Duvivier, D.H., Votion, D., Vandenput, S., Art, T., Lekeux, P., 1997. Airway response of horses with COPD to dry powder inhalation of ipratropium bromide. *The Veterinary Journal* 154, 149–153.
- Erin, E.M., Leaker, B.R., Nicholson, G.C., Tan, A.J., Green, L.M., Neighbour, H., Zacharasiewicz, A.S., Turner, J., Barnathan, E.S., Kon, O.M., Barnes, P.J., Hansel, T.T., 2006. The effects of a monoclonal antibody directed against tumor necrosis factor- α in asthma. *American Journal of Respiratory and Critical Care Medicine* 174, 753–762.
- Fairbairn, S.M., Page, C.P., Lees, P., Cunningham, F.M., 1993. Early neutrophil but not eosinophil or platelet recruitment to the lungs of allergic horses following antigen exposure. *Clinical and Experimental Allergy* 23, 821–828.
- Ferrer, L., Alberola, J., Queralt, M., Brazis, P., Rabanal, R., Llenas, J., Puigdemont, A., 1999. Clinical anti-inflammatory efficacy of arofyl-line, a new selective phosphodiesterase-4 inhibitor, in dogs with atopic dermatitis. *Veterinary Record* 145, 191–194.
- Foster, A.P., Cunningham, F.M., 1998a. The pathogenesis and immunopharmacology of equine insect hypersensitivity. *Advances in Veterinary Dermatology* 3, 177–189.

- Foster, A.P., Cunningham, F.M., 1998b. Histamine-induced adherence and migration of equine eosinophils. *American Journal of Veterinary Research* 59, 1153–1159.
- Foster, A.P., McKelvie, J., Cunningham, F.M., 1998. The histamine-1 receptor antagonist chlorpheniramine inhibits antigen-induced cutaneous inflammatory responses in ponies with insect hypersensitivity. *Veterinary Record* 143, 189–193.
- Franchini, M., Gilli, U., Akens, M.K., Fellenberg, R.V., Bracher, V., 1998. The role of neutrophil chemotactic cytokines in the pathogenesis of equine chronic obstructive pulmonary disease (COPD). *Veterinary Immunology and Immunopathology* 66, 53–65.
- Franchini, M., Gill, U., von Fellenberg, R., Bracher, V.D., 2000. Interleukin-8 concentration and neutrophil chemotactic activity in bronchoalveolar lavage fluid of horses with chronic obstructive pulmonary disease following exposure to hay. *American Journal of Veterinary Research* 62, 1369–1374.
- Ghio, A.J., Mazan, M.R., Hoffman, A.M., Robinson, N.E., 2006. Correlates between human lung injury after particle exposure and recurrent airway obstruction in the horse. *Equine Veterinary Journal* 38, 362–367.
- Giguère, S., Viel, L., Lee, E., MacKay, R.J., Hernandez, J., Franchini, M., 2002. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. *Veterinary Immunology and Immunopathology* 85, 147–158.
- Gilger, B.C., Michau, T.M., Salmon, J.H., 2005. Immune-mediated keratitis in horses: 19 cases (1998–2004). *Veterinary Ophthalmology* 8, 233–239.
- Gosens, R., Zaagsma, J., Meurs, H., Halayko, A.J., 2006. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. *Respiratory Research* 7, 1–15.
- Gray, P.R., Derksen, F.J., Broadstone, R.V., Robinson, N.E., Johnson, H.G., Olson, N.C., 1992. Increased pulmonary production of immunoreactive 15-hydroxyeicosatetraenoic acid in an animal model of asthma. *American Review of Respiratory Disease* 145, 1092–1097.
- Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdottir, S., Zurbriggen, A., Jungi, T., Marti, E., 2006. Equine insect bite hypersensitivity: Immunoblot analysis of IgE and IgG subclass responses to *Culicoides nubeculosus* salivary gland extract. *Veterinary Immunology and Immunopathology* 113, 99–112.
- Homey, B., Steinhoff, M., Ruzicka, T., Leung, D.Y.M., 2006. Cytokines and chemokines orchestrate atopic skin inflammation. *Journal of Allergy and Clinical Immunology* 118, 178–189.
- Horohov, D.W., Beadle, R.E., Mouch, S., Pourciau, S.S., 2005. Temporal regulation of cytokine mRNA expression in equine recurrent airway obstruction. *Veterinary Immunology and Immunopathology* 108, 237–245.
- Ismail, T., McSharry, C., Boyd, G., 2006. Extrinsic allergic alveolitis. *Respirology* 11, 262–268.
- Ito, K., Lim, S., Caramori, G., Cosio, B., Chung, K.F., Adcock, I.M., Barnes, P.J., 2002. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. *Proceedings of the National Academy of Sciences USA* 99, 8921–8926.
- Iwasaki, T., Hasegawa, A., 2006. A randomized comparative clinical trial of recombinant canine interferon- γ (KT-100) in atopic dogs using antihistamine as control. *European Society of Veterinary Dermatology* 17, 195–200.
- Jackson, K.A., Stott, J.L., Horohov, D.W., Watson, J.L., 2004. IL-4 induced CD23 (Fc γ RII) up-regulation in equine peripheral blood mononuclear cells and pulmonary alveolar macrophages. *Veterinary Immunology and Immunopathology* 101, 243–250.
- Jacobi, A., Antoni, C., Manger, B., Schuler, G., Hertl, M., 2005. Infliximab in the treatment of moderate to severe atopic dermatitis. *Journal of the American Academy of Dermatology* 52, 522–526.
- Jost, U., Klukowska-Rötzler, J., Dolf, G., Swinburne, J.E., Ramseyer, A., Bugno, M., Burger, D., Blott, S., Gerber, V., 2007. A region on equine chromosome 13 is linked to recurrent airway obstruction in horses. *Equine Veterinary Journal* 39, 236–241.
- Kleiber, C., McGorum, B.C., Horohov, D.W., Pirie, R.S., Zurbriggen, A., Straub, R., 2005. Cytokine profiles of peripheral blood and airway CD4 and CD8 T lymphocytes in horses with recurrent airway obstruction. *Veterinary Immunology and Immunopathology* 104, 91–97.
- Kobayashi, M., Nasuhara, Y., Betsuyaku, T., Shibuya, E., Tanino, Y., Tanino, M., Takamura, K., Nagai, K., Hosokawa, T., Nishimura, M., 2004. Effect of low dose theophylline on airway inflammation in COPD. *Respirology* 9, 249–254.
- Kolm, G., Zappe, H., Schmid, R., Riedelberger, K., Van den Hoven, R., 2003. Efficacy of montelukast in the treatment of chronic obstructive pulmonary disease in five horses. *Veterinary Record* 152, 804–806.
- Kolm, G., Knapp, E., Wagner, R., Klein, D., 2006. Increased interleukin-1 β mRNA expression in skin biopsies of horses with *Culicoides* hypersensitivity following challenge with *Culicoides nubeculosus* extract. *Veterinary Immunology and Immunopathology* 113, 90–98.
- Künzle, F., Gerber, V., Van Der Haegen, A., Wampfler, B., Straub, R., Marti, E., 2007. IgE-bearing cells in bronchoalveolar lavage fluid and allergen-specific IgE levels in sera from RAO-affected horses. *Journal of Veterinary Medicine A. Physiology, Pathophysiology and Clinical Medicine* 54, 40–47.
- Laan, T.T., Bull, S., Pirie, R.S., Fink-Gremmels, J., 2006a. The anti-inflammatory effects of IV administered clenbuterol in horses with recurrent airway obstruction. *Veterinary Journal* 171, 429–437.
- Laan, T.T., Bull, S., Pirie, R., Fink-Gremmels, J., 2006b. The role of alveolar macrophages in the pathogenesis of recurrent airway obstruction in horses. *Journal of Veterinary Internal Medicine* 20, 167–174.
- Larsson, K., Larsson, B-M., Sandström, T., Sundblad, B-M., Palmberg, L., 2001. Sodium cromoglycate attenuates pulmonary inflammation without influencing bronchial responsiveness in healthy subjects exposed to organic dust. *Clinical and Experimental Allergy* 31, 1356–1368.
- Lavoie, J.-P., Maghni, K., Desnoyers, M., Taha, R., Martin, J.G., Hamid, Q.A., 2001. Neutrophilic airway inflammation in horses with heaves is characterised by a Th2-type cytokine profile. *American Journal of Respiratory and Critical Care Medicine* 164, 1410–1413.
- Lavoie, J.-P., Léguillette, R., Pasloske, K., Charette, L., Sawyer, N., Guay, D., Murphy, T., Hickey, G.J., 2002. Comparison of effects of dexamethasone and the leukotriene D4 receptor antagonist, L-708,738 on lung function and airway cytologic findings in horses with recurrent airway obstruction. *American Journal of Veterinary Research* 63, 579–585.
- Lavoie, J.-P., Thompson, D., Hamilton, E., Debrue, M., David, F., Hickey, G., 2004. Comparison of dexamethasone and the MAPK p38 inhibitor MRL-1EQ in equine heaves. In: *Proceedings of the 22nd Symposium of the Veterinary and Comparative Respiratory Society*.
- Lavoie, J.-P., Pasloske, K., Joubert, P., Cordeau, M.-E., Mancini, J., Girard, Y., Friesen, R.W., Frenette, R., Blouin, M., Young, R.N., Hickey, G., 2006. Lack of clinical efficacy of a phosphodiesterase-4 inhibitor for treatment of heaves in horses. *Journal of Veterinary Internal Medicine* 20, 175–181.
- Léguillette, R., 2003. Recurrent airway obstruction – heaves. *The Veterinary Clinics Equine Practice* 19, 63–86.
- Levy, B.D., Bonnans, C., Silverman, E.S., Palmer, L.J., Marigowda, G., Israel, E., 2007. Diminished lipoxin biosynthesis in severe asthma. *American Journal of Respiratory and Critical Care Medicine* 172, 824–830.
- Lindberg, A., Robinson, N.E., Näsman-Glaser, B., Jensen-Waern, M., Lindgren, J.A., 2004. Assessment of leukotriene B $_4$ production in leukocytes from horses with recurrent airway obstruction. *American Journal of Veterinary Research* 65, 289–295.
- Lombart, H.-G., Feyfant, E., Joseph-McCarthy, D., Huang, A., Lovering, F., Sun, L.H., Zhu, Y., Zeng, C., Zhang, Y., Levin, J., 2007. Design and synthesis of 3,3-piperide hydroxamate analogs as selective TACE inhibitors. *Bioorganic and Medicinal Chemistry Letters*. E-pub May 16th.
- Marinkovic, D., Aleksic-Kovacevic, S., Plamenac, P., 2007. Cellular basis of chronic obstructive pulmonary disease in horses. *International Journal of Cytology* 257, 213–247.

- Marr, K.A., Lees, P., Page, C.P., Cunningham, F.M., 1998a. Inhaled leukotrienes cause bronchoconstriction and neutrophil accumulation in horses. *Research in Veterinary Science* 64, 219–224.
- Marr, K.A., Lees, P., Page, C.P., Cunningham, F.M., 1998b. Effect of the 5-lipoxygenase inhibitor, fenleuton, on antigen-induced neutrophil accumulation and lung function changes in horses with chronic obstructive pulmonary disease. *Journal of Veterinary Pharmacology and Therapeutics* 21, 241–246.
- Marr, K., Marsh, K., Hernandez, L., Cunningham, F.M., Lees, P., 1998c. Pharmacokinetics and pharmacodynamics of fenleuton, a 5-lipoxygenase inhibitor, in ponies. *Research in Veterinary Science* 64, 111–117.
- Marr, K.A., Lees, P., Cunningham, F.M., 2002. Antigen challenge increases adherence of circulating neutrophils in horses with chronic obstructive pulmonary disease. *Equine Veterinary Journal* 34, 65–70.
- Marti, E., Gerber, V., Jungi, T., Bertoni, G., 2007. Cytokine mRNA expression in skin biopsies of horses affected with insect bite hypersensitivity. Third Havemeyer Workshop on Allergic diseases of the horse, Iceland.
- McGorum, B.C., Dixon, P.M., Halliwell, R.E., 1993. Quantification of histamine in plasma and pulmonary fluids from horses with chronic obstructive pulmonary disease, before and after 'natural (hay and straw) challenges'. *Veterinary Immunology and Immunopathology* 36, 223–227.
- McKiernan, B.C., Koritz, G.D., Scott, J.S., Berney, C., Robinson, N.E., 1990. Plasma theophylline concentration and lung function in ponies with recurrent obstructive lung disease. *Equine Veterinary Journal* 22, 194–197.
- Molet, S., Gosset, P., Lassalle, P., Czarlewski, W., Tonnel, A.B., 1997. Inhibitory activity of loratidine and descarboxyethoxyloratidine on histamine-induced activation of endothelial cells. *Clinical and Experimental Allergy* 27, 1167–1174.
- Nelson, H.S., 2003. Prospects for antihistamines in the treatment of asthma. *Journal of Allergy and Clinical Immunology* 112, S96–S100.
- Newton, R., Holden, N.S., Catley, M.C., Oyelusi, W., Leigh, R., Proud, D., Barnes, P.J., 2007. Repression of inflammatory gene expression in human pulmonary epithelial cells by small-molecule I κ B kinase inhibitors. *Journal of Pharmacology and Experimental Therapeutics* 321, 734–742.
- O'Byrne, P.M., 2006. Cytokines or their antagonists for the treatment of asthma. *Chest* 130, 244–250.
- Oldhoff, J.M., Darsow, U., Werfel, T., Katzer, K., Wulf, A., Laifaoui, J., Hijnen, D.J., Plötz, S., Knol, E.F., Kapp, A., Bruijnzeel-Koomen, C.A.F.M., Ring, J., de Bruin-Weller, M.S., 2005. Anti-IL-5 recombinant humanized monoclonal antibody (Mepolizumab) for the treatment of atopic dermatitis. *Allergy* 60, 693–696.
- Olsén, L., Ingvast-Larsson, C., Larsson, P., Broström, H., Bondesson, U., Sundqvist, M., Tjälve, H., 2006. Fexofenadine in horses: pharmacokinetics, pharmacodynamics and effect of ivermectin pretreatment. *Journal of Veterinary Pharmacology and Therapeutics* 29, 129–135.
- Parkinson, J.F., 2006. Lipoxin and synthetic lipoxin analogues: An overview of anti-inflammatory functions and new concepts in immunomodulation. *Inflammation and Allergy – Drug Targets* 5, 91–106.
- Paus, R., Schmelz, M., Biro, T., Steinhoff, M., 2006. Frontiers in pruritus research: scratching the brain for more effective itch therapy. *Journal of Clinical Research* 116, 1174–1185.
- Perkins, C., Wills-Karp, M., Finkelman, F.D., 2006. IL-4 induces IL-13-independent allergic airway inflammation. *Journal of Allergy and Clinical Immunology* 118, 410–419.
- Pirie, R.S., Collie, D.D.S., Dixon, P.M., McGorum, B.C., 2003a. Inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (organic dust-induced asthma). *Clinical and Experimental Allergy* 33, 676–683.
- Pirie, R.S., Dixon, P.M., McGorum, B.C., 2003b. Endotoxin contamination contributes to the pulmonary inflammatory and functional response to *Aspergillus fumigatus* extract inhalation in heaves horses. *Clinical and Experimental Allergy* 33, 1289–1296.
- Pua, V.S.C., Barnetson, R.St.C., 2006. Recent developments in the treatment of adult atopic dermatitis. *Australasian Journal of Dermatology* 47, 84–89.
- Renz, H., Herz, U., 2002. The bidirectional capacity of bacterial antigens to modulate allergy and asthma. *European Respiratory Journal* 19, 158–171.
- Rickards, K.J., Page, C.P., Lees, P., Cunningham, F.M., 2000. Phosphodiesterase activity in neutrophils from horses with chronic obstructive pulmonary disease. *Veterinary Immunology and Immunopathology* 76, 319–330.
- Rickards, K.J., Page, C.P., Lees, P., Gettinby, G., Cunningham, F.M., 2003. In vitro and ex vivo effects of the PDE4 inhibitor, rolipram, on thromboxane production in equine blood. *Journal of Veterinary Pharmacology and Therapeutics* 26, 123–130.
- Rickards, K.R., Page, C.P., Hamblin, A.S., Goode, N.T., Cunningham, F.M., 2004a. Biochemical and functional assessment of equine lymphocyte phosphodiesterase and protein kinase C. *Veterinary Immunology and Immunopathology* 98, 153–165.
- Rickards, K.R., Page, C.P., Cunningham, F.M., 2004b. Allergen challenge alters lymphocyte phosphodiesterase activity in horses with heaves. *Pulmonary Pharmacology and Therapeutics* 17, 163–172.
- Riek, R.F., 1955. Studies on allergic dermatitis (Queensland itch) of the horse: The origin and significance of histamine in the blood and its distribution in the tissues. *Australian Journal of Agricultural Research* 6, 161–170.
- Robinson, N.E., 2001. Recurrent airway obstruction. In: Lekeux, P. (Ed.), *Equine Respiratory Diseases*, <http://www.avis.org/special_books/Lekeux/robinson/IVIS.pdf> (accessed 22.08.2007).
- Robinson, N.E., Derksen, F.J., Olszewski, M.A., Buechner-Maxwell, V.A., 1996. The pathogenesis of chronic obstructive pulmonary disease. *British Veterinary Journal* 152, 283–306.
- Sandersen, C., Bureau, F., Turlej, R., Fiévez, L., Dogné, S., Kirschvink, N., Lekeux, P., 2001. p65 Homodimer activity in distal airway cells determines lung dysfunction in equine heaves. *Veterinary Immunology and Immunopathology* 80, 315–326.
- Schäcke, H., Döcke, W.-D., Asadullah, K., 2002. Mechanisms involved in the side effects of glucocorticoids. *Pharmacology and Therapeutics* 96, 23–43.
- Schäcke, H., Schottelius, A., Döcke, W.-D., Strehlke, P., Jaroch, S., Schmees, N., Rehwinkel, H., Hennekes, H., Asadullah, K., 2004. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proceedings of the National Academy of Sciences USA* 101, 227–232.
- Schmallenbach, K.H., Rahman, I., Sasse, H.H.L., Dixon, P.M., Halliwell, R.E.W., McGorum, B.C., Cramer, R., Miller, H.R.P., 1998. Studies on pulmonary and systemic *Aspergillus fumigatus*-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Veterinary Immunology and Immunopathology* 66, 245–256.
- Stanford, J.L., 2007. Progress with the treatment of sweet itch. In: *Sweet Itch News*, Spring, 2007.
- Steffan, J., Favrot, C., Mueller, R., 2006. A systematic review and meta-analysis of the efficacy and safety of cyclosporine for the treatment of atopic dermatitis in dogs. *Veterinary Dermatology* 17, 3–16.
- Sullivan, P., Bekir, S., Jaffar, Z., Page, C., Jeffery, P., Costello, J., 1994. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma [published erratum appears in *Lancet* 1994 June 11th, 343(8911):1512]. *Lancet* 343, 1006–1008.
- Tanigawa, K., Nagase, H., Ohmori, K., Tanaka, K., Miyake, H., Kaniwa, M., Ikizawa, K., 2002. Species-specific differences in the glucocorticoid receptor transactivation function upon binding with betamethasone-esters. *International Immunopharmacology* 2, 941–950.
- Thomson, J.R., McPherson, E.A., 1981. Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse. *Equine Veterinary Journal* 13, 243–246.
- Van den Hoven, R., Duvigneau, J.C., Hartl, R.T., Gemeiner, M., 2006. Clenbuterol affects the expression of mRNA for interleukin 10 in peripheral leukocytes from horses challenged intrabronchially with lipopolysaccharides. *Veterinary Research Communications* 30, 921–928.

- Van der Haegen, A., Griot-Wenk, M., Welle, M., Busato, A., von Tschärner, C., Zurbriggen, A., Marti, E., 2001. Immunoglobulin-E-bearing cells in skin biopsies of horses with insect bite hypersensitivity. *Equine Veterinary Journal* 33, 699–706.
- Wagner, B., Miller, W.H., Morgan, E.E., Hillegas, J.M., Erb, H.N., Leibold, W., Antczak, D.F., 2006a. IgE and IgG antibodies in skin allergy of the horse. *Veterinary Research* 37, 813–825.
- Wagner, B., Hillegas, J.M., Antczak, D.F., 2006b. A monoclonal antibody to equine interleukin-4. *Veterinary Immunology and Immunopathology* 110, 363–367.
- Weston, M.C., Collins, M.E., Cunningham, F.M., 2006a. Equine CCL11 induces eosinophil cytoskeletal reorganisation and activation. *Inflammation Research* 55, 46–52.
- Weston, M.C., Cunningham, F.M., Collins, M.E., 2006b. Cloning of equine CCR3 – mRNA expression in horse tissues. *Veterinary Immunology and Immunopathology* 114, 238–246.
- Wijnker, J.J., Bull, S., van Dijk, P., Veenman, J.N., Rutten, V.P., Klein, W.R., Fink-Gremmels, J., 2004. Modulation of cytokine responses in equine macrophages following TACE-inhibition. *Veterinary Immunology and Immunopathology* 99, 237–243.
- Wills-Karp, M., 2004. Interleukin-13 in asthma pathogenesis. *Immunological Reviews* 202, 175–190.
- Wilson, A.D., Harwood, L.J., Björnsdóttir, S., Marti, E., Day, M.J., 2001. Detection of IgG and IgE serum antibodies to *Culicoides* salivary gland antigens in horses with insect dermal hypersensitivity (sweet itch). *Equine Veterinary Journal* 33, 707–713.
- Wilson, A.D., Harwood, L., Torsteinsdóttir, S., Marti, E., 2006. Production of monoclonal antibodies specific for native equine IgE and their application to monitor total serum IgE responses in Icelandic and non-Icelandic horses with insect bite dermal hypersensitivity. *Veterinary Immunology and Immunopathology* 112, 156–170.
- Woodfolk, J.A., 2006. Cytokines as a therapeutic target for allergic diseases: a complex picture. *Current Pharmaceutical Design* 12, 2349–2363.
- Yamagata, T., Ichinose, M., 2006. Agents against cytokine synthesis or receptors. *European Journal of Pharmacology* 533, 289–301.