REVIEW

The $K^+$ channels $K_{Ca3.1}$ and $K_V1.3$ as novel targets for asthma therapy

Peter Bradding$^1$ and Heike Wulff$^2$

$^1$Department of Infection, Immunity and Inflammation, Institute for Lung Health, University of Leicester, UK, and $^2$Department of Pharmacology, University of California, Davis, CA, USA

Asthma affects 10% of the UK population and is an important cause of morbidity and mortality at all ages. Current treatments are either ineffective or carry unacceptable side effects for a number of patients; in consequence, development of new approaches to therapy are important. Ion channels are emerging as attractive therapeutic targets in a variety of non-excitable cells. Ion channels conducting $K^+$ modulate the activity of several structural and inflammatory cells which play important roles in the pathophysiology of asthma. Two channels of particular interest are the voltage-gated $K^+$ channel $K_V1.3$ and the intermediate conductance $Ca^{2+}$-activated $K^+$ channel $K_{Ca3.1}$ (also known as $I_{KCa1}$ or $SK4$). $K_V1.3$ is expressed in IFN-$\gamma$-producing T cells while $K_{Ca3.1}$ is expressed in T cells, mast cells, macrophages, airway smooth muscle cells, fibroblasts and epithelial cells. Both channels play important roles in cell activation, migration, and proliferation through the regulation of membrane potential and calcium signalling. We hypothesize that $K_{Ca3.1}$- and/or $K_V1.3$-dependent cell processes are one of the common denominators in asthma pathophysiology. If true, these channels might serve as novel targets for the treatment of asthma. Emerging evidence lends support to this hypothesis. Further validation through the study of the role that these channels play in normal and asthmatic airway cell (patho)physiology and in vivo models will provide further justification for the assessment of small molecule blockers of $K_V1.3$ and $K_{Ca3.1}$ in the treatment of asthma.

Keywords: asthma; $K_{Ca3.1}$; $K_V1.3$; ion channel

Abbreviations: ASM, airway smooth muscle; ACD, allergic contact dermatitis; BHR, bronchial hyperresponsiveness; EAE, experimental autoimmune encephalomyelitis; HLMC, human lung mast cell

Asthma pathophysiology

Asthma affects 10% of westernized populations and is an important cause of morbidity and mortality at all ages (Masoli et al., 2004; Asher et al., 2006). It is a complex disease characterized by airway inflammation, airway wall remodelling and bronchial hyperresponsiveness (BHR). Exactly how these three key features interact and whether they are dependent on each other for their occurrence remain unknown. There is continued debate about the most important cell type mediating the airway changes in asthma, but critical analysis of the current evidence indicates that most if not all elements of the asthmatic airway are dysfunctional. There is epithelial dysfunction with failure of healing and overproduction of growth factors and pro-inflammatory cytokines (Holgate et al., 1999), mucous gland hyperplasia with associated mucus hypersecretion (Carroll et al., 2002), airway smooth muscle (ASM) dysfunction with resulting hypertrophy, hyperplasia, BHR and cytokine secretion (Ebina et al., 1990; 1993; Brightling et al., 2005), and inflammatory cell activation with ‘over-active’ mast cells (Bradding et al., 2006), T cells (Robinson et al., 1992), eosinophils (Bradding et al., 1994), and neutrophils (Carroll et al., 2002). The current cornerstone of asthma management is the use of inhaled corticosteroids, which are efficacious in about 90% of patients (Barnes and Adcock, 2003). However, for approximately 10% of patients, steroids are of poor efficacy for reasons that are not understood. These severe or refractory patients are difficult to treat, suffer great morbidity and use up a disproportionate fraction of healthcare resources (Wenzel, 2005). Novel treatments for asthma targeting the inflammatory response are emerging, but to date, these have been disappointing. An example is the use of anti-TNF$\alpha$ strategies, which, although promising in small pilot studies, have proved ineffective in larger randomized controlled trials (Berry et al., 2006; Wenzel et al., 2009). Similar disappointment has occurred with the use of anti-interleukin (IL)-4 (O’Byrne, 2006). There is therefore an unmet clinical need for new asthma drugs with different mechanisms of action and/or adverse-effect profiles.
The K⁺ channels K₁.3 and KC₃.1 as potential novel therapeutic targets for asthma

Cells such as muscle and nerves fire action potentials and are known as excitable cells. The role of ion channels in propagating these electrical impulses is well described. In contrast, cells that do not have fire action potentials such as leukocytes are generally regarded as non-excitable cells. However, molecular biology and patch-clamp analyses in recent years have shown that non-excitable cells such as lymphocytes express a complex mix of ion channels carrying K⁺, Cl⁻, Ca²⁺ and non-selective combinations of cations (Chandy et al., 2004; Bradding, 2005). These channels are expressed at different levels depending on the cell subset and the state of activation and differentiation. Influx of extracellular Ca²⁺ is an essential requirement for the activity of many cellular processes (Berridge et al., 2000). K⁺ channels play an important role in Ca²⁺ signalling through their ability to maintain a negative membrane potential during cell activation (Ghanshani et al., 2000; Fanger et al., 2001; Duffy et al., 2004), which enhances Ca²⁺ influx through inward-rectifier Ca²⁺ channels due to an increased electrical driving force for Ca²⁺ entry (Hoth and Penner, 1992). For example, in T cells (Figure 1), the voltage-gated K⁺ channel K₁.3 and the Ca²⁺-activated K⁺ channel KC₃.1 regulate Ca²⁺ influx through the calcium-release activated Ca²⁺ channel, which consists of the Ca²⁺-sensor stromal interaction molecule 1 and the pore-forming protein CRACM1 (Orai1) (Zhang et al., 2005; Prakriya et al., 2006; Prakriya et al., 2006; Vig et al., 2006; Veromyn et al., 2006; Lis et al., 2007). The Ca²⁺ influx results in the increase in cytosolic Ca²⁺ concentration necessary for the translocation of nuclear factor of activated T cells (NFAT) to the nucleus and the initiation of new transcription, ultimately resulting in cytokine secretion and T cell proliferation (Dolmetsch et al., 1997; 1998; Lewis, 2001). However, this crucial influx of Ca²⁺ is only possible if the T cell can keep its membrane potential negative by a counterbalancing K⁺ efflux through K₁.3 and/or KC₃.1 (Lin et al., 1993; Chandy et al., 2004). Both channels are therefore regarded as attractive new targets for immunosuppression (Chandy et al., 2004).

In addition to T cells, K₁.3 and KC₃.1 are widely distributed amongst immune and structural airway cells, where they play key roles in cellular activation, proliferation and migration by regulating membrane potential and Ca²⁺ signalling processes. We therefore hypothesise that KC₃.1- and/or K₁.3-dependent cell processes are one of the common denominators in asthma pathophysiology. If true, these channels might serve as novel targets for the treatment of asthma.

K₁.3

Both K₁.3 and KC₃.1 have a well-developed pharmacology and have been shown previously to be amenable to drug therapy. Functional K₁.3 channels are opened by membrane depolarization, with half maximal opening occurring at ~40 mV to ~35 mV (Cahalan et al., 1985; Grissmer et al., 1990). With cell depolarization, a conformational change moves the voltage sensor in the S4 transmembrane domain and opens the channel pore (Larsson et al., 1996). There are several potent and relatively selective inhibitors of K₁.3. These include ShK (K₈ 11 pM), a 35-amino acid polypeptide derived from the Caribbean Sea anemone Stichodactyla helianthus, and margatoxin (K₈ 110 pM), which is derived from the scorpion Centruroides margaritatus (Chandy et al., 2004). Both bind to the outer mouth of the channel and physically obstruct ion conduction. Once bound, their dissociation is very slow so that their effects may persist for several hours. The specificity of ShK for K₁.3 is greatly enhanced by the substitution of the critical Lys²⁵ in ShK with diaminopropionic acid (ShK-Dap²⁵) (Kalman et al., 1998) or by attachment of 1-phosphorytoxosine to the N-terminus (ShK(L5)) (Beeton et al., 2005). These analogues are remarkably stable in cell culture systems and in vivo. PAP-1 [5-(4-phenoxybutoxy)psoralen] is the first relatively specific small molecule blocker of K₁.3 (K₈ 2 nm) (Schmitz et al., 2005). A further useful tool for the study of K₁.3 is a fluorescein-6-carboxylic acid (F6CA)-labelled analogue of ShK. F6CA-ShK binds with high affinity to K₁.3 channels and can be used to detect them in T cells using flow cytometry (Beeton et al., 2003).

K₃.1

KC₃.1 channels have a similar topological structure to K₁.3, but rather than containing a voltage sensor in the S4 domain, they bind calmodulin tightly near the C-terminus, which serves as the Ca²⁺ sensor. KC₃.1 channels are thus opened by a rise in cytosolic free Ca²⁺ [Ca²⁺]i] due to Ca²⁺-calmodulin-mediated cross-linking of subunits in the channel tetramer (Fanger et al., 1999). Channel function is reported to be increased by membrane-associated protein kinase A through phosphorylation of either the channel protein itself or a closely associated accessory protein in oocytes and T84 cells (Gerlach et al., 2000). In CD4⁺ T cells, KC₃.1 activity is increased by the nucleoside diphosphate kinase B, which phosphorylates KC₃.1 on histidine 358 (Srivastava et al., 2006). In contrast, histidine 358 is dephosphorylated by the mammalian protein histidine phosphatase, which directly binds to the KC₃.1 protein and negatively regulates T cell Ca²⁺ flux by decreasing KC₃.1 activity (Srivastava et al., 2008). KC₃.1 modulation in T cells is thus one of the rare examples of histidine phosphorylation/dephosphorylation influencing a biological process in mammals.

There are several tools for the study of KC₃.1 function. Charybdotoxin is a 37-amino acid peptide isolated from the venom of the scorpion Leiurus quinquestriatus and blocks KC₃.1 with a K₈ of 5 nM but also blocks the large conductance K⁺ channel KC₃.1 (BKᵥ₄.1) and K₁.3 with similar potency (Chandy et al., 2004; Wulff et al., 2007). Another more potent but less commonly used peptidic KC₃.1 blocker is maurotoxin (K₈ 1 nM) from the venom of the Tunisian scorpion Scorpio maurus (Khrara et al., 1996; Castle et al., 2003). In contrast to charybdotoxin, maurotoxin does not affect K₃.1 but instead potently inhibits the voltage-gated K₃.2 channel (K₈ 100 pM). Structural modification of the azole antimycotic clotrimazole (K₈ 70–250 nM) has resulted in the generation of the small molecule TRAM-34, which specifically blocks KC₃.1.
with a $K_d$ of 20 nM. TRAM-34 blocks $K_{Ca,3.1}$ by binding to internal residues below the selectivity filter, in contrast to charybdotoxin, which binds to the external pore (Wulff et al., 2000). ICA-17043 ($K_d$ 11 nM) is another small molecule blocker with high specificity for $K_{Ca,3.1}$ (Stocker et al., 2003). Interestingly, $K_{Ca,3.1}$ channels can be activated by a number of benzimidazolones and benzothiazoles, which increase the $Ca^{2+}$ sensitivity of these $Ca^{2+}$/calmodulin-gated channels. 

Figure 1 Involvement of $K_v1.3$, $K_{Ca,3.1}$ and CRAC (Orai 1) in the activation of a T cell by an antigen-presenting cell. Engagement of the T-cell receptor–CD3 complex through an antigenic peptide presented in the context of major histocompatibility complex (MHC) class II leads to the activation of phospholipase C$_{y}$ (PLC$_{y}$) downstream of the tyrosine kinases LCK and ZAP70. PLC$_{y}$ catalyses the hydrolysis of the membrane phospholipid PIP$_2$ to inositol-1,4,5-triphosphate (IP$_3$) and diacylglycerol. IP$_3$ opens the IP$_3$ receptor (IP$_3$R) in the membrane of the endoplasmic reticulum (ER), resulting in the release of $Ca^{2+}$ from intracellular stores. The rise in intracellular $Ca^{2+}$ activates the phosphatase calcineurin, which then dephosphorylates the transcription factor NFAT, enabling it to translocate to the nucleus and to bind to the promoter of cytokine genes such as interleukin 2 (IL-2). CRAC, $K_v1.3$ and $K_{Ca,3.1}$ critically regulate $Ca^{2+}$ signalling. Depletion of internal $Ca^{2+}$ stores is ‘sensed’ by the EF-hand containing stromal interaction molecule 1 (STIM1), which redistributes and clusters into sites adjacent to the plasma membrane and activates CRAC channels. The ensuing $Ca^{2+}$ influx through CRAC channels depolarizes the T cell and reduces $Ca^{2+}$ entry through the ‘inward’-rectifier CRAC. The driving force for $Ca^{2+}$ entry is restored by membrane hyperpolarization brought about by the opening of $K_v1.3$ channels in response to membrane depolarization and the opening of $K_{Ca,3.1}$ channels in response to $Ca^{2+}$ binding to calmodulin (CAM). (The resting intracellular $Ca^{2+}$ concentration in T cells is 50–100 nM and rises to about 1 $\mu$M during T cell activation. The extracellular $Ca^{2+}$ concentration is 1–2 mM).
‘classic’ activator 1-ethyl-2-benzimidazolone activates heterologously expressed KCa3.1 with an EC50 of 30 μM and achieves maximal potassium currents at 100 μM in the presence of 100 nM free Ca2+, which is below the resting (Ca2+)i of most cell types (Pedersen et al., 1999). A more potent KCa3.1 activator is the recently described benzothiazole SKA-31 [naphtho(1,2-d)thiazol-2-ylamine], which activates KCa3.1 with an EC50 of 250 nM (Sankaranarayanan et al., 2009). The structures of PAP-1, TRAM-34, ICA-17043, 1-EBIO and SKA-31 are shown in Figure 2, and the selectivity of TRAM-34, PAP-1 and SKA-31 is shown in Table 1.

### Table 1 The relative ion channel selectivity of TRAM-34, PAP-1 and SKA-31

<table>
<thead>
<tr>
<th>Channel</th>
<th>TRAM-34</th>
<th>PAP-1</th>
<th>SKA-31</th>
</tr>
</thead>
<tbody>
<tr>
<td>K+1</td>
<td>K+1.1</td>
<td>9.5 μM</td>
<td>65 nM</td>
</tr>
<tr>
<td>K+1.2</td>
<td>4.5 μM</td>
<td>250 nM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>K+1.3</td>
<td>5 μM</td>
<td>2 nM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>K+1.4</td>
<td>7.5 μM</td>
<td>75 nM</td>
<td>n.d.</td>
</tr>
<tr>
<td>K+1.5</td>
<td>7 μM</td>
<td>45 nM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>K+1.6</td>
<td>n.d.</td>
<td>62 nM</td>
<td>n.d.</td>
</tr>
<tr>
<td>K+3</td>
<td>K+3.1</td>
<td>30 μM</td>
<td>3 μM</td>
</tr>
<tr>
<td>K+3.2</td>
<td>n.d.</td>
<td>1 μM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>K+4.2</td>
<td>6 μM</td>
<td>1.2 μM</td>
<td>&gt;50 μM</td>
</tr>
<tr>
<td>K+11</td>
<td>K+11.1</td>
<td>20 μM</td>
<td>5 μM</td>
</tr>
<tr>
<td>Na+</td>
<td>Na+2.1</td>
<td>&gt;20 μM</td>
<td>15 μM</td>
</tr>
<tr>
<td>Ca+</td>
<td>Ca+2.1</td>
<td>25 μM</td>
<td>2.5 μM</td>
</tr>
<tr>
<td>Ca+2.2</td>
<td>20 μM</td>
<td>10 μM</td>
<td>3 μM*</td>
</tr>
<tr>
<td>Ca+2.3</td>
<td>20 μM</td>
<td>5 μM</td>
<td>2 μM*</td>
</tr>
<tr>
<td>Ca+3.1</td>
<td>20 μM</td>
<td>10 μM</td>
<td>250 nM*</td>
</tr>
<tr>
<td>Na+</td>
<td>Na+1.2</td>
<td>20 μM</td>
<td>7 μM</td>
</tr>
<tr>
<td>Na+1.4</td>
<td>7 μM</td>
<td>7 μM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>Na+1.5</td>
<td>n.d.</td>
<td>10 μM</td>
<td>&gt;25 μM</td>
</tr>
<tr>
<td>Ca+</td>
<td>Ca+1.2</td>
<td>12 μM</td>
<td>5 μM</td>
</tr>
</tbody>
</table>

Values marked by an asterisk (*) are EC50 values for channel activation. All other values are IC50 values for channel inhibition. n.d., not done.

Kv, voltage gated K+ channels; KIR, inwardly rectifying K+ channels; KCa, Ca2+-activated K+ channels; Na+, voltage gated Na+ channels; Ca+, voltage gated Ca2+ channels. For further information on ion channel nomenclature see Alexander et al. (2008).

**Cellular expression and function of Kv1.3 and KCa3.1**

In this section we will give a brief summary of what is currently known about the expression and (patho)physiological function of K+1.3 and KCa3.1 in T cells, mast cells, epithelial cells, ASM cells, and fibroblasts.

**T cells**

Human T cells express both K+1.3 and KCa3.1. However, the relative expression of the two channels depends on the activation and differentiation states of the cells and correlates with the expression of the chemokine receptor CCR7 and the phosphatase CD45RA. In the resting state, CCR7+CD45RA- naïve T cells, CCR7+CD45RA- central memory T cells (Tcm) and CCR7+CD45RA- effector memory T cells (Tem) in both the CD4+ and the CD8 compartment express ~250 K+1.3 and less than 20 KCa3.1 channels per cell (Beeton et al., 2003; Wulff et al., 2003). Following activation, naïve and Tem cells transcriptionally up-regulate KCa3.1 to 500 channels per cell without any change in K+1.3 expression (Figure 3). In contrast, CCR7+ Tem cells exclusively increase K+1.3 expression to approximately 1500 channels per cell following activation. This differential expression of K+1.3 and KCa3.1 in CCR7+ versus CCR7- T cells has important functional consequences. Naïve and Tem cells are initially affected by K+1.3 blockers but quickly become insensitive to them because they up-regulate KCa3.1 during activation and then rely on KCa3.1 for proliferation and cytokine secretion (Ghanshani et al., 2000; Wulff et al., 2003). In contrast, CCR7- Tem cells solely rely on K+1.3
for their activation processes, and Kv1.3 blockers like ShK(L5) and PAP-1 potently inhibit their Ca\(^{2+}\) flux following TCR ligation, and their IFN\(\gamma\), IL-2 and IL-17 production as well as their proliferation (Beeton et al., 2006; Azam et al., 2007). Kv1.3 blockers have therefore been proposed for the selective suppression of T\(_{EM}\) cells, while Kv\(_{\alpha\beta\gamma}\) blockers are regarded as more useful for immune responses that are carried by CCR7\(^{+}\) naïve and T\(_{EM}\) cells. In pre-activated T cells, Kv\(_{\alpha\beta\gamma}\) channels are localized evenly throughout the T cell plasma membrane, but rapidly redistribute to the immunological synapse following antigen presentation, where they co-localize with CD3 and F-actin (Nicolaou et al., 2007). Similar findings have been reported in T\(_{EM}\) cells for Kv1.3, synapse-associated protein 97, ZIP (PKC\(\xi\)-interacting protein, p56\(\xi\)-associated p62 protein), p56\(\xi\) and CD4 (Beeton et al., 2006).

Whether a Kv1.3 or a Kv\(_{\alpha\beta\gamma}\) blocker would be more useful for suppressing T cells in asthmatic airways is currently not clear because both Th1 cells (Krug et al., 1996) (which are presumably of a T\(_{EM}\) phenotype) and Th2 cells (which have been reported to express high levels of Kv\(_{\alpha\beta\gamma}\) (Fanger et al., 2000) are implicated in the immunopathology of asthma (Robinson et al., 1992). The fact that Kv1.3 blockers strongly inhibit the IL-2 and IFN\(\gamma\) production of T cells from the synovial fluid of patients with RA but have little effect on IL-4 and TNF\(\alpha\) production (Beeton et al., 2006) might suggest that Kv1.3 is not an ideal target in asthma. However, there is good evidence of IFN\(\gamma\) over-expression by asthmatic T cells (Krug et al., 1996; Brightling et al., 2002) and of activation of Th1-dependent pathways such as the CXCR3/CXCL10 axis (Miotto et al., 2001; Brightling et al., 2005).

**Mast cells**

While Kv1.3 is not expressed in human or mouse mast cells, we have identified Kv\(_{\alpha\beta\gamma}\) expression in human lung, blood-derived and bone marrow-derived mast cells (Duffy et al., 2001, 2004; Kaur et al., 2005). In addition, Shumilina et al. (2008) have described the presence of Kv\(_{\alpha\beta\gamma}\) in mouse bone marrow-derived mast cells. Kv\(_{\alpha\beta\gamma}\) channels open following IgE-dependent activation (Duffy et al., 2001, 2005; Kaur et al., 2005) resulting in acute plasma membrane hyperpolarization (Figure 4) and enhanced Ca\(^{2+}\) influx from the extracellular fluid, but with no effect on Ca\(^{2+}\) release from internal stores (Duffy et al., 2001; 2004; Shumilina et al., 2008). In consequence, block of Kv\(_{\alpha\beta\gamma}\) channels in human lung mast cells (HLMCs) with charybdotoxin attenuates HLMC histamine release in response to IgE-dependent activation (Duffy et al., 2001). Similarly, in mouse bone marrow-derived mast cells cultured from Kv\(_{3.1}\) knockout mice, degranulation in response to IgE-dependent activation is reduced by ~50%, although IL-6 secretion is not affected (Shumilina et al., 2008). Because secretion is only partially dependent on channel opening, Kv\(_{3.1}\) can be considered to increase the gain of an immunological stimulus. Although histamine release is not completely abrogated by Kv\(_{3.1}\) knockout, the Kv\(_{3.1}\) knockout mouse nevertheless has less severe systemic anaphylactic reactions (Shumilina et al., 2008), indicating that this is biologically relevant.

The growth of bone marrow-derived mast cells in Kv\(_{3.1}\) knockout mice or HLMC in the presence of Kv\(_{3.1}\) blockers TRAM-34 and charybdotoxin (ChTX), but not the Kv1.1 blocker ibenoxotin (IbTX) (B), n = 4 donors. *P < 0.05, **P < 0.01. ASM S/N-dependent migration in (A) is represented as 100% in (B). Dimethyl sulfoxide (DMSO) 0.1% was present in all conditions. Reproduced from Cruse et al. (2006).

We have observed that Kv\(_{3.1}\) is regulated in HLMC by the \(\beta_{2}\)-adrenoceptor (Duffy et al., 2005), the adenosine \(A_{2}\) receptor (Duffy et al., 2007) and the EP\(_{2}\) prostanoid receptor (Duffy et al., 2008). The effects occur rapidly and are not modulated by analogues of cAMP or forskolin, suggesting that they occur through a \(G_{i}\)-coupled membrane-delimited mechanism (Duffy et al., 2005). Activation of these receptors closes Kv\(_{3.1}\), which may explain in part how they inhibit both mast cell secretion and migration (Gebhardt et al., 2005; Duffy et al., 2007; 2008).
Epithelium

The airway epithelium is at the interface with the external environment and is the first structure to interact with noxious stimuli such as allergens, viruses, and pollutants. Not only does the columnar epithelium tend to shed from the basal layer, the airway epithelium is also functionally abnormal in asthma (Holgate et al., 1999; Puddicombe et al., 2000). Epithelial repair normally involves up-regulation of the epidermal growth factor (EGF) receptor, which drives the repair response. In asthmatic epithelium, the proliferative repair response is impeded, but other consequences of EGF receptor activation remain intact. Thus, there is ongoing release of pro-inflammatory cytokines which may promote cellular recruitment, and there is release of profibrogenic growth factors which may drive the remodelling response (Holgate et al., 1999; Puddicombe et al., 2000). Both K\textsubscript{1.3} and K\textsubscript{Ca3.1} are expressed by epithelial cell lines (Devor et al., 1999; Grunnet et al., 2003). In particular, K\textsubscript{Ca3.1} expression has been reported in Calu-3 cells (Devor et al., 1999). The proposed role for K\textsubscript{Ca3.1} in epithelium is to reduce HCO\textsubscript{3}\textsuperscript{-} secretion and to increase Cl\textsuperscript{-} secretion (Devor et al., 1999). We predict that K\textsubscript{Ca3.1} will contribute to the secretion of pro-inflammatory cytokines and mucus by epithelial cells through its ability to potentiate Ca\textsuperscript{2+} influx.

ASM and fibroblasts

The central physiological abnormality in asthma is BHR, which results in airflow obstruction in response to bronchospastic stimuli (Boushey et al., 1980; Boulet, 2003). The ASM in asthma is therefore highly dysfunctional, and in addition demonstrates both hypertrophy and hyperplasia (Ebina et al., 1990; 1993). Whether the ASM in asthma is fundamentally different to that in normal subjects due to either genetic or acquired factors is not known. However, in vitro several profound phenotypic differences are evident (Johnson et al., 2001; 2004; Burgess et al., 2003; Both et al., 2004; Brightling et al., 2005). We were the first to demonstrate that K\textsubscript{Ca3.1} is expressed by both normal and asthmatic human ASM (Shepherd et al., 2007). K\textsubscript{Ca3.1} expression is increased by both basic fibroblast growth factor (FGF) and TGF\textbeta, and K\textsubscript{Ca3.1} inhibition with TRAM-34 attenuates human ASM proliferation (Shepherd et al., 2007). This up-regulation of K\textsubscript{Ca3.1} in ASM is reminiscent of the K\textsubscript{Ca3.1} up-regulation that occurs in mouse, rat, and pig vascular or coronary smooth muscle during the remodelling associated with restenosis and atherosclerosis (Kohler et al., 2003; Tharp et al., 2006; 2008; Toyama et al., 2008). We envisage that K\textsubscript{Ca3.1} mediates important biological effects in the ASM of asthmatic subjects and that K\textsubscript{Ca3.1} blockade might at least partially prevent ASM remodelling.

Fibroblasts, specifically myofibroblasts, contribute to the deposition of collagen beneath the airway epithelium in asthma (Brewster et al., 1999). Fibroblast cell lines express a K\text sub{Ca} channel with the biophysical properties of K\textsubscript{Ca3.1} (Rane, 1991; Penas and Rane, 1999), and charybdo toxin prevents FGF-induced fibroblast proliferation. Whether primary human airway fibroblasts express K\textsubscript{Ca3.1} has not been reported, however, we believe it is highly likely that K\textsubscript{Ca3.1} plays an important role in the fibrogenic activity of human airway fibroblasts.

Other cells

K\textsubscript{Ca3.1} is also expressed by other cells of potential importance to asthma. Human endothelial cell expression of K\textsubscript{Ca3.1} was increased by both basic FGF and VEGF, two growth factors implicated in the angiogenesis which characterizes human asthma (Shute et al., 2004; Siddiqui et al., 2007). Blockade of K\textsubscript{Ca3.1} with charybdo toxin and TRAM-34 inhibited human endothelial cell proliferation in vitro, while TRAM-34 inhibited angiogenesis in mice in an in vivo matrigel plug assay (Grgic et al., 2005). Inhibition of K\textsubscript{Ca3.1} may therefore be expected to prevent or reverse the angiogenesis evident in asthmatic airways.

Macrophages have also been implicated in asthma, although their role remains poorly defined (Holgate, 2008). K\textsubscript{Ca3.1} is expressed by human and mouse macrophages, and K\textsubscript{Ca3.1} knockout or pharmacological inhibition has been shown to suppress macrophage activation and migration (Schmid-Antonarchi et al., 1997; Toyama et al., 2008). K\textsubscript{Ca3.1} has not been described to date in eosinophils.

Roles in disease

Pharmacological blockers of both K\textsubscript{1.3} and K\textsubscript{Ca3.1} have been tested in many disease models. Compounds that block K\textsubscript{1.3} suppress T\textsubscript{H1} function in vitro and effectively treat memory T cell-mediated immune reactions such as delayed-type hypersensitivity (DTH) in rats and minipigs (Kos et al., 1997; Beeton et al., 2005; Schmitz et al., 2005), as well as experimental autoimmune encephalomyelitis (EAE) (Beeton et al., 2001), experimental autoimmune diabetes (Beeton et al., 2006), pristane-induced arthritis (Beeton et al., 2006) and allergic contact dermatitis (ACD) in rats (Azam et al., 2007), without causing any toxic side effects (Beeton et al., 2006). In all these disease models K\textsubscript{1.3} blockers seem to have selectively suppressed the adenosine signaling in vivo imaging study, which showed that K\textsubscript{1.3} blockers inhibited DTH and suppressed T\textsubscript{H1} cell enlargement and motility in inflamed tissue but had no effect on homing to or motility in lymph nodes of naive and central memory T cells (Matheu et al., 2006). In keeping with this observation, K\textsubscript{1.3} blockers did not prevent antigen presentation and memory T cell development in oxazoline-induced ACD in rats but effectively inhibited ear swelling during the T\textsubscript{H1} cell-mediated effector phase of the disease (Azam et al., 2007). K\textsubscript{Ca3.1} blockers that inhibit the activation and migration of naïve T cells, and many structural and inflammatory cells in vitro, have been shown to treat EAE in mice and to prevent vascular restenosis after systemic delivery in rats (Kohler et al., 2003) and after local delivery in pigs (Tharp et al., 2008). The K\textsubscript{Ca3.1} blocker TRAM-34 further reduces atherosclerosis development in ApoE-/- mice by inhibiting both vascular smooth muscle cell proliferation and T cell and macrophage activity (Toyama et al., 2008). Of relevance to asthma, the K\textsubscript{Ca3.1} knockout mouse displays an attenuated IgE-dependent systemic anaphylactic response (Shumilina et al., 2008). Furthermore, it is reported on the websites of the pharmaceutical companies Icagen Inc (Durham, NC, USA) that the orally active K\textsubscript{Ca3.1} blocker ICA-17043 (Senicapoc) inhibits the late airway response and the development of BHR following

allergen challenge in a sheep model of asthma (http://www.icagen.com/randd/memorydisorders.html).

Safety of targeting K\textsubscript{1.3} and KCa3.1

K\textsubscript{1.3}

A key issue for any long-term therapy is a favourable balance between efficacy and safety.

In addition to CCR7 T\textsubscript{EM} cells, K\textsubscript{1.3} is also expressed in the central nervous system, kidney, liver, skeletal muscle, platelets, macrophages, testis and osteoclasts, raising the possibility that K\textsubscript{1.3} blockers could have adverse side effects. To investigate this possibility, the Wulff and Chandy laboratories performed 28-day and 6-month toxicity studies with PAP-1 (50 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} orally) and a 28-day toxicity study with ShK-L5 (500 µg·kg\textsuperscript{-1}·day\textsuperscript{-1} s.c.) in both male and female rats (Beeton et al., 2006). (Please note that PAP-1 effectively prevents autoimmune diabetes in diabetes-prone BB/W or rats at the same dose and that Shk-L5 suppresses DTH at 10 µg·kg\textsuperscript{-1} and treats EAE at 100 µg·kg\textsuperscript{-1}). Both blockers failed to induce any histopathological changes in any tissue examined, including those reported to express K\textsubscript{1.3}. PAP-1 and Shk-L5 also did not induce any changes in haematological or serum chemistry parameters. Both blockers further did not delay influenza virus clearance in rats, suggesting that K\textsubscript{1.3} blockers truly selectively inhibit T\textsubscript{EM} cells and do not affect the function of naïve and T\textsubscript{CM} cells (Matheu et al., 2008). In collaboration with Dr Aftab Ansari at the Primate Center of Emory University, the Wulff laboratory also administered PAP-1 at 25 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} for 28 days to rhesus macaques. The treatment again did not induce any changes in blood chemistry or haematology and did not affect the development of a protective T\textsubscript{CM} response following nasal flu vaccination (Pereira et al., 2007). However, in keeping with a role of T\textsubscript{CM} cells in suppressing chronic viral infections, PAP-1 treatment caused a reactivation of CMV virus, which however, did not result in any symptoms of CMV disease but was detectable by PCR. Before performing these experiments we thoroughly tested PAP-1 for in vitro toxicity and found that it is not cytotoxic, not phototoxic, and is negative in the Ames test, which assesses mutagenic potential. Most importantly, PAP-1 exhibits excellent selectivity over other ion channels as well as various receptors and transporters (Schmitz et al., 2005). The relative safety of K\textsubscript{1.3} blockers may be due in part to channel redundancy and also because K\textsubscript{1.3} blockers may not inhibit K\textsubscript{1.3}-containing heteromultimers (e.g. in the central nervous system (CNS)) with the same affinity as K\textsubscript{1.3} homotetramers in T cells.

KCa3.1

Similar to K\textsubscript{1.3}, KCa3.1 seems to be relatively safe as a therapeutic target. Two independently generated K\textsubscript{Ca3.1}−/− mice (Begenisich et al., 2004; Si et al., 2006) were both viable, of normal appearance, produced normal litter sizes, did not show any gross abnormalities in any of their major organs and exhibited rather mild phenotypes: impaired volume regulation in erythrocytes and lymphocytes (Begenisich et al., 2004), a reduced endothelial-derived hyperpolarising factor (EDHF) response together with a mild −7-mmHg increase in blood pressure (Si et al., 2006), and subtle erythrocyte macrocytosis and progressive splenomegaly (Grbic et al., 2009). Pharmacological blockade of K\textsubscript{Ca3.1} also seems to be safe and well tolerated. TRAM-34 exhibits an excellent selectivity over other ion channels and was ‘clean’ in a Hit Profiling screen on 32 neuronal receptors and transporters (Wulff et al., 2000; Toyama et al., 2008). Daily administration of TRAM-34 at 120 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} did not induce any changes in blood chemistry, haematology or necropsy of major organs in a 28-day toxicity study in mice or rats (Toyama et al., 2008). There have also been no reports about toxicity for the structurally related K\textsubscript{Ca3.1} blocker ICA-17043 (Senicapoc), which was developed by Icagen Inc. and which entered clinical trials as an orphan drug for sickle cell anemia (Stacker et al., 2003). ICA-17043 was found to be both effective and safe in Phase-2 clinical trials (Ataga et al., 2008), but the phase-III trials were stopped in 2007 due to a lack of efficacy in reducing sickling crises. ICA-17043 recently re-entered clinical trials and is currently being evaluated for asthma in two phase-II proof-of-concept trials. Dose-escalating studies with ICA-17043 in 28 otherwise healthy patients with sickle cell disease did not increase blood pressure or lead to electrocardiogram changes (Ataga et al., 2006; 2008).

Summary

In summary, K\textsubscript{1.3} and KCa3.1 regulate many diverse cell processes of relevance to asthma. As such, they offer the potential for the development of a truly novel approach to the treatment of this disease. Further validation of these targets is required to define which aspects of the asthmatic process are most likely to be attenuated by K\textsubscript{1.3} or KCa3.1 blockade in humans. In turn, this will help determine the primary outcomes for clinical trials. For example, if eosinophilia is the predominant feature that is inhibited, then the rate of exacerbations should be the primary outcome (Green et al., 2002), whereas if BHR or remodelling is the predominant feature that improves, then measurement of these as the primary outcome would be more appropriate. The studies to date with K\textsubscript{1.3} and KCa3.1 blockers are encouraging, and the lack of any toxicity with ICA-17043 when administered to humans with sickle cell disease or of TRAM-34 and PAP-1 administered to rodents and primates suggests real therapeutic potential for human disease.

Conflicts of interest

Peter Bradding has undertaken contract research and acted as a consultant for Icagen Inc. Heike Wulff is an inventor on the University of California-owned patents claiming TRAM-34 and PAP-1 as immunosuppressants. Her laboratory has received student fees from Icagen Inc., and she is co-founder of Airmid Inc, a company aiming to develop K\textsubscript{1.3} blockers for the treatment of multiple sclerosis and psoriasis.

References


