ABSTRACT

Ion channels are specialized transmembrane proteins that permit the passive flow of ions following their electrochemical gradients. In the airways, ion channels participate in the production of epithelial-based hydroelectrolitic secretions and in the control of intracellular Ca\(^{2+}\) levels that ultimately will activate almost all lung cells, either resident or circulating cells. Thus, ion channels have been the centre of many studies aiming to understand asthma pathophysiological mechanisms or to identify therapeutical targets for better control of the disease. We will focus this review on the molecular, genetic and animal models studies associating ion channels with asthma.

Asthma is an inflammatory disorder of the conducting airways characterized by generalized reversible obstruction of the airflow that affects between 1-18% of the population depending on the country (1). Asthma etiology is complex and multifactorial in which both a hereditary component (one or more containing genetic variations that enhance susceptibility) and the environment participate (2,3). The chronic inflammation is associated with bronchial hyperresponsiveness (BHR) that leads to recurrent episodes of shortness of breath, cough and wheezing. At the
pathophysiological level, asthma results from complex biological interactions between different cell types, both resident (i.e., epithelial and smooth muscle cells) and circulating cells (mainly immune cells), with environmental factors such as allergens, infections and tobacco smoke (1,4). A key element in this pathophysiological process is the T lymphocyte (T_{H2}) that orchestrates chronic inflammation, smooth muscle contraction and airway remodeling (3,4). Another key feature is a defective airway epithelium, easing allergen contact with mucosal antigen-presenting dendritic cells (DCs), which in turns will promote a T_{H2} phenotype (5,6). Other immune cells such as B lymphocytes, mast cells and eosinophils as well as sensory neurons innervating the airways and endothelial cells involved in vascular permeation also participate (7-10).

Ion channels regulate many key functions of the cells implicated in asthma pathophysiology (Figure 1). Therefore, intense research on the channels contribution to the genesis or therapy of the disease has been carried out over the last 30 years. Similar to asthma pathogenesis, that has moved from an intrinsic airway smooth muscle abnormality through an autonomous nervous system dysfunction to the present-day inflammatory disorder, the role of ion channels in asthma has also evolved. The initial interest on ion channels was classically centered on their role on airways smooth muscle (ASM) contraction. Following the identification of voltage-gated calcium channels (VGCC) responsible for smooth and cardiac muscle contraction and their pharmacological inhibition in the 70’s (11), these channels capitalized early asthma studies (12,13). They were followed by the potassium channels that modify membrane potential and, consequently, the activation of VGCC in smooth muscle (14,15). Chloride channels, due to their crucial involvement in many airway epithelial functions and smooth muscle contraction (16-19) have also appeared recurrently in asthma studies. Nowadays, the focus has moved away from ASM channels toward those involved in sensing irritants or the inflammatory response, particularly the non-selective cationic Transient Receptor Potential (TRP) channels (20,21).

Additional support for the role of ion transport in the pathogenesis of asthma has recently and unexpectedly come in the form of a genetic association study. A genome-wide association study of childhood asthma showed the strongest, and almost exclusive, association with the ORMDL3 gene (22). The product of this gene is an endoplasmic reticulum (ER) protein that participates in ER-mediated Ca^{2+} homeostasis and stress responses (23).

There are many channels analyzed in airways cells, the function of which may contribute to the disorder but due to the short
format of this review we will primarily focus on those ion channels whose association with asthma pathogenesis or its clinical manifestations has been evaluated in molecular, genetic or animal models studies.

EPITHELIAL ION CHANNELS

Early observations carried out in asthmatic patients revealed the presence of a damaged epithelium (24) that may facilitate the permeability of the airways to inhaled irritants, allergens and pathogens as well as the exposure of sensory nerves and the release of inflammatory mediators. Currently, it is postulated that the allergen sensitization may well be the consequence of a defective airway epithelium (5,6) leading to inappropriate programming of mucosal DC cells (25,26). An important factor that contributes to an impaired barrier function is the presence of defective epithelial tight junction (TJ) formation or epithelial repairing mechanisms. Both processes appear to be influenced by ion transport systems that may work independently of their transport function (27,28). In the airways, several ion channels have been linked to TJ formation, epithelial permeability or repair: the cystic fibrosis transmembrane conductance regulator (CFTR) (29,30), Kv7.1 (KCQ1), Kir6.1 (KATP) and KCa3.1 (KCNN4) potassium channels (31). Other channels that are also expressed in airway epithelia although their role in epithelial barrier or repairing functions have been demonstrated elsewhere include: CIC2 (32), TRPC1 (33), TRPV4 (34) and TRPC4 (35). Considering that these ion channel-dependent cell processes are common denominators in asthma pathophysiology, their study -either measuring function or expression levels- in asthmatic airways or in animal models may provide novel insights into the pathogenesis of the disorder.

The neuronal sensory TRPV1 channel (the founding member of the vanilloid subfamily of TRP channels (36)) has also been detected in immortalized human airways epithelial cells lines and implicated in the particulate matter-induced apoptosis (37), thereby affecting the integrity of the epithelial barrier. However, no response to capsaicin, the classical TRPV1 activator, has been observed in native mouse tracheal epithelial cells (Figure 2). It would be interesting to test whether native human airway epithelium expresses functional TRPV1 channels. TRPM8, a member of the TRPM subfamily (Melastatin) that functions as a cold transducer in the somatosensory system (38,39), mediates cold-dependent increased transcription of epithelial cytokine and chemokine genes (40) and, therefore, may participate in the cold-induced aggravation of respiratory symptoms and asthma (41).

Other functions of conducting airway epithelia related to hydroelectrolitic transport,
osmo-mechanical responses and mucociliary clearance are also linked to the activity of ion channels and/or intracellular calcium signaling (16,42-47). Of particular interest for airways pathophysiology are the CFTR Cl⁻ channel and the epithelial Na⁺ channel (ENaC). Mutations in the CFTR gene results in cystic fibrosis (CF), a disease characterized by altered Cl⁻ and Na⁺ channel activity that results in airways mucus obstruction, infection and inflammation (48). CFTR and ENaC channels participate in fluid secretion and reabsorption thereby controlling the volume and composition of the airway surface liquid (ASL), which in turns affects cilia beating and mucociliary clearance (49). Defects in airways cilia (structural or functional) affect the incidence of respiratory infection, but the presence of primary mucociliary dysfunction in asthmatics is still a matter of debate, probably being more relevant to chronic obstructive pulmonary disease (COPD) (50). Transgenic βENaC mouse models resume many characteristics of airway inflammatory response in the absence of pathogens (51) and reduced expression of all ENaC subunits have been found in preterm infants with respiratory distress (52). To date there is no evidence for a direct association between ENaC or CFTR malfunctioning with asthma, apart for one study that associates several CFTR mutations with asthma, although those mutations were also found in healthy individuals and subsequent studies did not support the original findings (53). Other airway epithelial channels have also been the subject of genetic epidemiological studies. A loss-of-function single nucleotide polymorphism (SNP) (54) in the TRPV4 channel involved in ciliary beating frequency regulation (46,55) have shown no association with asthma (56) but was associated with COPD (57) and hyponatremia (54).

**AIRWAY SMOOTH MUSCLE ION CHANNELS**

Airway smooth muscle (ASM) controls airflow through the conducting airways. Its contraction reduces airflow while relaxation facilitates it. ASM plays a central role in bronchial hyperresponsiveness and remodeling (58) and has being the subject of intense research to identify the molecular mechanisms participating in its contraction, proliferation and migration. Ion channels facilitating ASM contraction aim to increase intracellular overall Ca²⁺ concentration (e.g., VGCC (59)) while those favoring bronchodilatation generally produce the opposite effect (e.g., potassium channels (60)). The role of ion channels in ASM contraction and asthma pathophysiology have been critically reviewed (61,62) and the initial emphasis on VGCC blockers and potassium channel openers has not been warranted by their success in clinical trials ((14,63) and references within).
ASM present voltage-dependent L-type (Ca\textsubscript{v}1.1) and T-type (Ca\textsubscript{v}3.2) Ca\textsuperscript{2+} channels (59). Activation of L-type channel following membrane depolarization -and its interplay with ryanodine receptors in the endoplasmic reticulum- triggers an increase in [Ca\textsuperscript{2+}] and ASM contraction. Interestingly, the γ regulatory subunit of the L-type channel (CACNG6) that stabilizes the inactivation of the channel, has been recently associated with aspirin-intolerance asthma in a Korean population (64).

Potassium channels contribute to the relaxation of ASM by hyperpolarizing the membrane potential and, thereby, preventing the activation of voltage-gated Ca\textsuperscript{2+} channels. Electrophysiological and molecular approaches have facilitated the identification of several K+ channels in ASM (although for some only indirect evidence exists): Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (K\textsubscript{Ca}), voltage-activated K\textsuperscript{+} channels (K\textnu) and ATP-sensitive K\textsuperscript{+} channels (K\textsubscript{ATP}) (65-68). Despite their clear contribution to ASM physiology, evidences for their involvement in asthma pathophysiology are scant. Loss-of-function SNPs of the \( \beta_1 \) regulatory subunit (KCMB1) of the pore forming \( \alpha \) subunit of the voltage- and Ca\textsuperscript{2+}-activated large conductance K\textsuperscript{+} channel (K\textsubscript{Ca}1.1, KCNN4 and also known as BK) has been associated with asthma severity in African Americans (69). However, a BK deficient mouse model presented an unexpected reduced, rather than increased, ASM contractility due to a compensatory up-regulation of the eGMP pathway, which may reflect the important role of BK channels in ASM contraction (70). BK channel impact on ASM relaxation has received further support from a very recent study showing that bitter tastants activate BK and relax the airways of a asthma mouse model with higher efficacy than the currently used \( \beta \)-agonists (71). K\textsubscript{Ca}3.1 channel (also known as KCNN4 or IK\textsubscript{Ca}), in addition to regulate ASM contraction is also implicated in ASM proliferation, being up-regulated by TGF-\( \beta \), a regulatory process that is more pronounced in asthmatics (66). Pharmacological inhibition of K\textsubscript{Ca}3.1 prevents proliferation of ASM (66,72) and modulates the function of K\textsubscript{Ca}3.1-expressing immune cells (see following sections). Another ASM K\textsuperscript{+} channel relevant to asthma pathophysiology is the KCNS3, a non-conducting \( \alpha \) subunit K\textsubscript{9.3} with a regulatory function on K\textsubscript{2.1} (KCNN1) channels. Different SNPs in KCNS3 have been associated with airway hyperresponsiveness, although no functional dysregulation has been proven (73).

Several TRP channels have also been identified in ASM (20,74) but only those contributing to BHR and/or remodeling will be discussed. Most TRP channels are non-selective channels that mediate intracellular Ca\textsuperscript{2+} increases either directly or via membrane despolarization and activation of
VGCC. The TRPC1 channel contributes to ASM proliferation (75), and presumably airway thickening, while TRPC3 and TRPC6 channels main role relates to ASM contraction (76,77). Besides, TRPC3 expression in ASM increases in the ovalbumin (OVA)-sensitized asthmatic mouse model (76) and in response to the proinflammatory cytokine TNF-α (78), which rises the question of whether the efficacy of TNF-α antagonists in the treatment of asthma (79) may also involve TRPC3.

ION CHANNELS IN IMMUNE CELLS
As in many other cells, ion channels in immune cells mainly aim to control cytosolic Ca\(^{2+}\) signals, which in turn, will regulate short (i.e., mast cell degranulation) and long term cellular responses (i.e., T cell proliferation and cytokine production) (80). Particularly relevant is the Ca\(^{2+}\) entry mechanism (the calcium release activated current, CRAC (81)) triggered by the crosslinking of antigen receptors, activation of phospholipase-C/inositol trisphosphate (IP\(_3\)) pathway and the subsequent depletion of endoplasmic reticulum (ER) Ca\(^{2+}\) stores. This event, named store-operated Ca\(^{2+}\) entry (SOCE), relies on two recently discovered elements, the ER Ca\(^{2+}\) sensor STIM that communicates to the plasma membrane Ca\(^{2+}\) channel Orai the need to replenish the intracellular store (82). Considering the key role played by immune cells in asthma pathogenesis and that their activation is typically link to SOCE mechanisms, it is surprising the few studies focusing on SOCE in the context of immune cell function in asthma. Blocking CRAC prevents T\(_{H2}\) mediated responses in a murine model of asthma (83) while mast cells derived from Stim1-KO and Orai1-KO mice present defective degranulation and activation of transcription factors NFAT and NF-κB (84,85).

The function of many other ion channels in immune cells is principally to regulate CRAC current by modulating the driving force for calcium entry through Orai channels. Potassium channel activation hyperpolarizes the cell membrane potential thereby favoring Ca\(^{2+}\) entry via channels other than VGCC while K\(^{+}\) channel inhibition prevents it. Both voltage-dependent (K\(_{V1.3}\)) and Ca\(^{2+}\)-dependent (K\(_{Ca3.1}\)) K\(^{+}\) channels regulate T cell activation and proliferation (86,87), and the latter has also been involved in mast cells IgE mediated histamine release (88).

TRP channels are involved in different immune cells function with relevance to asthma pathophysiology. TRPC6-KO mice show reduced airway eosinophilia, blood IgE levels and T\(_{H2}\) cytokines (IL-5, IL-13), resulting in decreased allergic airways response (77). The Ca\(^{2+}\)-activated nonselective cation channel TRPM4 contributes to membrane depolarization thereby reducing SOCE due to
a smaller Ca^{2+} driving force after FceR1 stimulation of mast cells or chemokines in the case of DC. Thus, TRPM4-KO mice show increased SOCE with a more severe IgE-mediated acute passive response (89) and altered migration of DC (90).

To finish with this section it is worth mentioning the unexpected, but interesting, role of Ca_{v}1.2 in T_n2 cytokine production and development of airway inflammation. Besides, knocking-down Ca_{v}1.2 ameliorates the asthma induced in murine models (91).

ION CHANNELS IN SENSORY NERVES
Nerves innervating the lung control different aspects of the airway physiology: gland secretions, epithelial transport, dilation of vessels and ASM contraction. Nerves also mediate different reflex responses, cough and sneezing, aiming to protect the airways from chemical and biological challenges (92). The vagus nerve provides most of the nerves innervating the airways (sensory and parasympathetic nerves) whereas sympathetic innervation comes from the spinal cord. Most important for asthma pathophysiology and several of its manifestations are the sensory nerves whose cells bodies are located in the nodose, jugular and dorsal root ganglia. Abnormal neuronal function may contribute to airway disease. Stimulation of sensory terminals triggers protective reflex responses that when occurring at the lower airways may even produce bronchoconstriction and neurogenic inflammation by the release of inflammatory mediators. TRP channels are implicated in the detection and initiation of reflex responses to chemicals and postulated to play a role in the pathogenesis of chronic respiratory diseases. TRPV1 activity has been related to neurogenic inflammation (93), irritant-induced chronic cough (94) and airways hypersensitivity (95). Besides, a loss of function mutation in TRPV1 associates with lower risk of presenting wheezing and cough in asthmatic children (56). Another TRP channel that has received considerable attention in recent times is TRPA1, as this channel appears to mediate the airways response to many different toxic gases and irritants, including cigarette smoke (96), nicotine (97), oxidants (98), heavy metals (99) and general anesthetics (100). TRPA1 activation evokes coughing in animal models and humans (101) and, more impressively, TRPA1-KO mice show an alleviation of the inflammatory processes triggered by allergens in the OVA model of asthma (102).

CONCLUSIONS
Asthma is a disorder presenting dysfunctional elements at all cellular levels in the airways and ion channels regulate one way or another, the function of all airways cells. The emphasis of ion channel research in asthma has been for a long time centered on ASM and immune cells channels, but is now shifting towards the sensory channels of the
nerves. Although ASM channel pharmacology has not been effective to date, the challenge now is to use the ion channels recently identify as key elements in asthma pathogenesis and responses to environmental factors as targets for the development of new pharmacological tools for novel and improved treatments.

REFERENCES


FOOTNOTES

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The abbreviations used are: ASM, airways smooth muscle; CFTR, cystic fibrosis transmembrane conductance regulator; COPD, chronic obstructive pulmonary disease; DC, dendritic cells; ER, endoplasmic reticulum; ENaC, epithelial ion channel; OVA, ovalbumin; SNP, single nucleotide polymorphism; SNP, single nucleotide polymorphism; SOCE, store-operated Ca$^{2+}$ entry; TJ, tight junction; TRP, Transient receptor potential cation channels; VGCC, voltage-gated calcium channels

FIGURE LEGENDS
Figure 1. Ion channels and asthma. Schematic overview of the different airways cells showing the ion channels associated with asthma pathophysiology or its clinical symptoms. See text for a detailed explanation.
Figure 2. Calcium responses to activators of TRPV1 and purinergic receptors in mouse tracheal ciliated cells. Average calcium increases measured with the Ca\(^{2+}\)-sensor Fura-2 in a primary culture of mouse tracheal cells exposed to two different concentrations (100 nM and 1 µM) of the TRPV1 activator capsaicin. Under these conditions ciliated epithelial cells did not respond to capsaicin, but responded to ATP (20 µM), a typical physiological activator of purinergic receptors. Results are expressed as the mean±SE of 10 cells.