



Involvement of ion channels in allergy

Lucette Pelletier and Magali Savignac

Allergic asthma is a complex disease, often characterized by an inappropriate Th2 response to normally harmless allergens. Epithelial cells damaged or activated by the allergen produce IL-33, TSLP and IL-25, activating ILC2 and dendritic cells. The latter migrate into lymph nodes where they induce Th2-cell commitment. Th2 and other type 2 innate inflammatory cells trigger inflammation and airway hyper-reactivity. The toolbox consisting of the ion channels varies from one cellular type to another and depends on its activation state, offering the possibility to design novel drugs in the field of allergy. We will discuss about some channels as calcium, nonselective cation, potassium and chloride channels that appear as good candidates in allergy.

Address

Center of Physiopathology Toulouse Purpan, University Paul Sabatier
Toulouse III, INSERM U1043, CNRS UMR 5282, 31024 Toulouse, France

Corresponding author: Pelletier, Lucette (Lucette.Pelletier@inserm.fr)

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Introduction

Allergy is initiated by an inadequate immune responsiveness to normally harmless airway allergens, occurring in genetically predisposed humans. The allergic diseases include atopic dermatitis, allergic asthma, and food allergies. We will focus on allergic asthma that affects more than 300 millions of people worldwide. This chronic pulmonary disease often starts during childhood and causes airway hyper-responsiveness [1•].

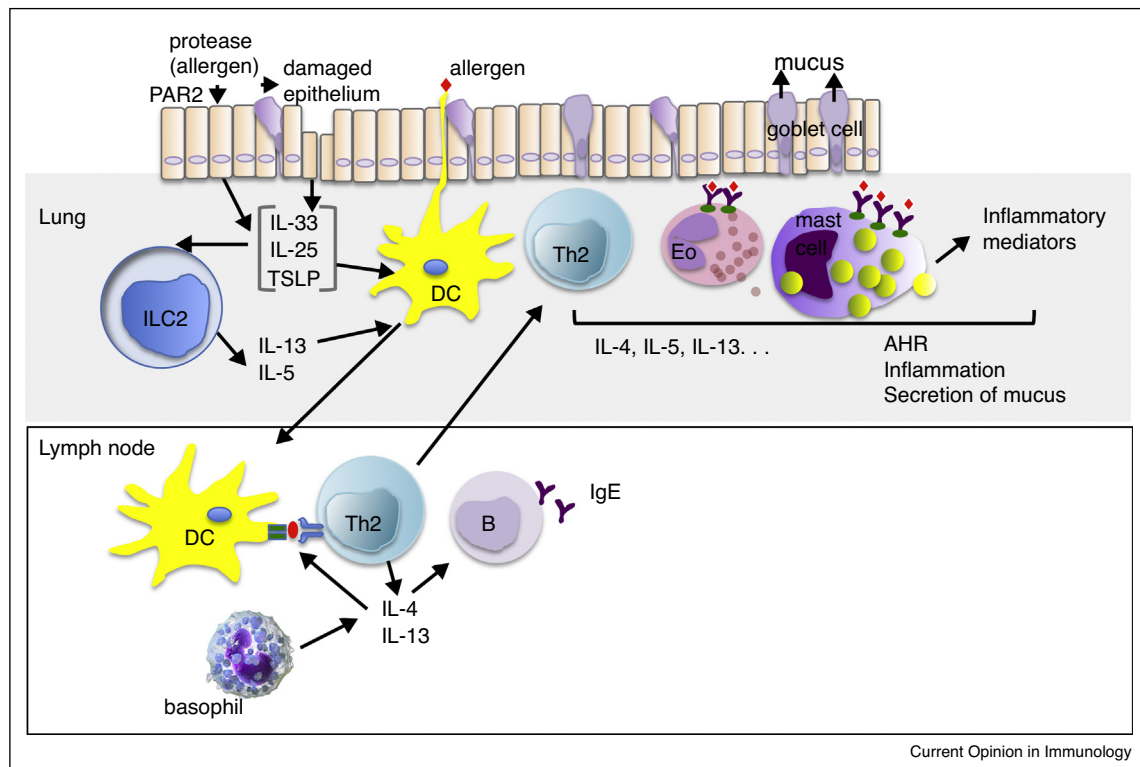
Antigen recognition induces the differentiation of naive T-cells into T helper cell effectors that include several subsets as Th1, Th2, and Th17. These populations express master transcription factors, produce a specialized pattern of cytokines and exert distinct functions. Th2-cells express the master transcription factor GATA-3 and are normally devoted to the eradication of parasites. We focus on this subset that produces interleukins (IL)-4, 5,

9, 13 and GM-CSF [2,3] because their role in allergy has been well demonstrated in experimental models of allergic asthma but also in humans. Dendritic cells (DCs) pick up the allergen in the lungs, get activated, mature, and migrate into the draining lymph nodes where they induce Th2-cell commitment (Figure 1). IL-4 and IL-13 are implicated in immunoglobulin (Ig)E commutation switch by B-lymphocytes. IL-4 promotes Th2 differentiation through STAT-6 signaling and thus amplifies the pool of Th2-cells. Activated Th2-cells will migrate to the lungs where they contribute to inflammation. IL-5 promotes the differentiation of eosinophils (a hallmark of allergic diseases) and their recruitment into the lungs. IL-13 has a major impact on disease development (Figure 2) [4]. Indeed, it allows the recruitment of inflammatory cells into the lungs and acts on epithelial cells resulting in the production of chemo-attractants. IL-13 also enables the production of mucus by goblet cells, fosters bronchoconstriction and prevents NO-mediated muscle relaxation. It also acts on fibroblasts to promote TGFβ production and fibrosis.

An important issue is how Th2-cells are preferentially induced in allergic patients. There are several nonexclusive possible explanations. Allergy often develops in early infancy. At that time, lung structures are vulnerable to environmental injuries [5]. In addition, the immune system that is immature is skewed to Th2-response [6]. These factors contribute to the development of allergic asthma.

The nature of allergens is also of concern. Indeed, they have often protease activity, inducing epithelial damage and alteration of the epithelial barrier but they also target the production of chemokines, IL-25, 33 and TSLP by epithelial cells [7] (Figure 1). These cytokines have at least three roles. First, they instruct DCs to promote Th2-cell differentiation. One important clue is the expression of the cluster of differentiation CD134 (OX40 ligand) and the absence of production of IL-12 by DCs [8,9•]. Second, they activate type 2 innate lymphoid cells (ILC2) that express the transcription factors GATA-binding protein (GATA)-3 and the retinoic acid-related orphan receptor (ROR) α. ILC2 share many properties with Th2-cells [10]. On one hand, ILC2 favor DC migration to the draining lymph nodes through the production of IL-13 and the initiation of Th2 responses [11•]. On the other hand, ILC2 contribute to the recruitment of type-2 inflammatory cells in the lungs by producing IL-5 and IL-13. Third, interestingly, IL-33 can activate Th2-cells in a TCR-independent manner, which amplifies inflammation [12,13,14•]. Besides mast cells, basophils and

Figure 1



Scheme of asthma induction. Allergens have often protease activity, which can directly damage epithelial cells releasing IL-33. These proteases bind to protease-activated receptor (PAR-2) at the surface of epithelial cells leading to the production of IL-25 and thymic stromal lymphopoietin (TSLP). These mediators activate innate lymphoid cells (ILC)-2 and/or dendritic cells (DC). DCs that have picked up the allergen migrate to the lymph nodes where they instruct allergen-specific T-cells to become Th2-cells. IL-4 and IL-13 enable the commutation isotype switch to IgE by B-lymphocytes. Activated Th2-cells migrate to the tissue and together with innate inflammatory cells produce IL-4, IL-5, and IL-13 that are all deleterious in asthma. Mast cells and eosinophils (eo) can be activated through the binding of IgE on high affinity IgE receptors and crosslinking by the allergen. AHR: Airway hyper-reactivity.

eosinophils, NKT [15] and some macrophages can amplify allergic inflammation through the production of type 2 cytokines [16]. Of note, the disease evolves over time due to close interactions between the immune system and the lung tissue [7] promoting and sustaining inflammation and airway remodeling. Current treatments are mostly symptomatic even if the blockade of IL-4/IL-5 axis is currently proposed to stop Th2 inflammation [17,18]. Severe asthma is not associated with pure type-2 response. Thus a Th17 signature has been found in severe asthma [19], which could lead to propose Th2 and Th17-cells as therapeutic targets in some forms of asthma.

One interesting way to look for alternative therapies is to target ionic channels. First, ionic homeostasis is essential for the survival and functions of all the cells in basal and activated states. Second, this homeostasis is achieved through toolboxes containing different sets of ionic channels depending on the cell type. We will focus on some of the channels that have been tested at least in experimental asthma (Figure 3).

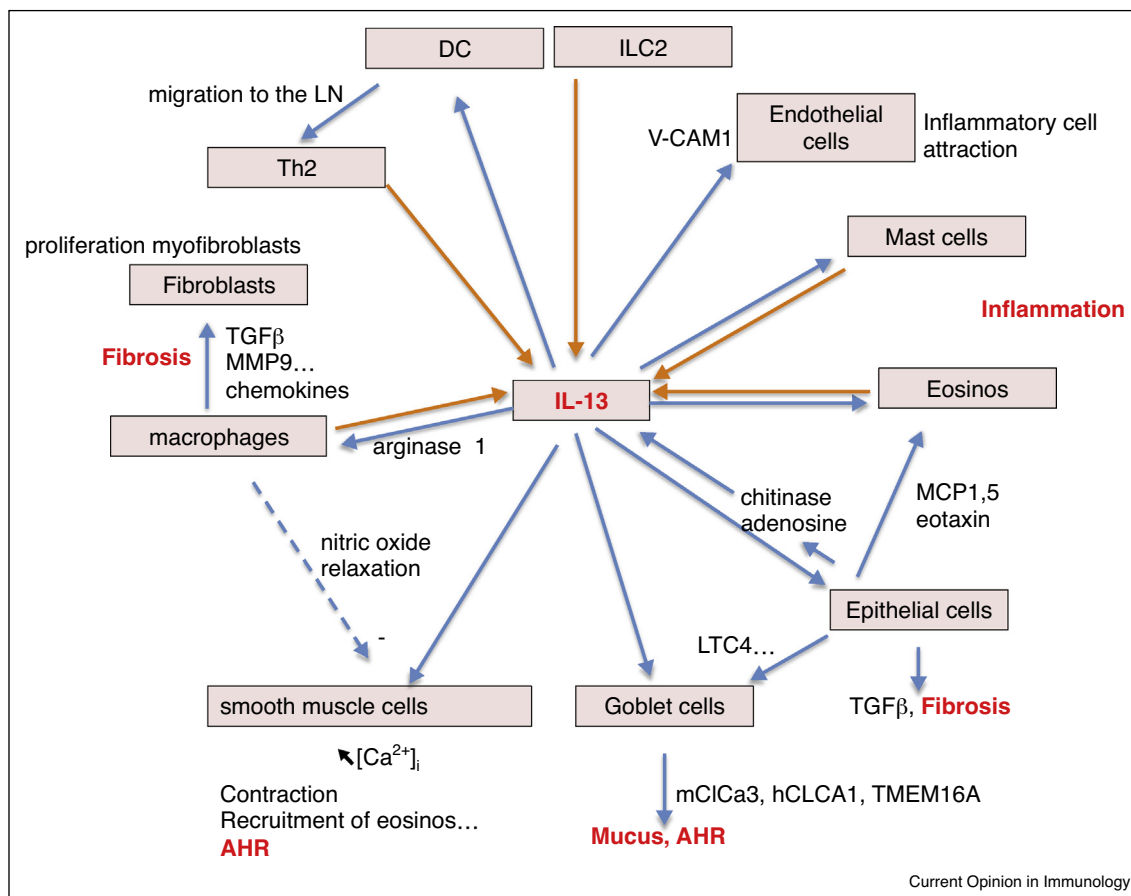
Calcium channels as a target in asthma

Calcium ions (Ca^{2+}) act as a second messenger governing the cellular fate. Indeed, binding of Ca^{2+} to EF hand-domains alters the properties of many proteins as structural proteins, enzymes (CaMK, PLC γ , and PKC), signaling molecules, molecules involved in contraction, exocytosis, membrane fusion, adhesion. Channels at the cell and ER plasma membrane let to increase intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). We will describe some of them, the inhibition of which is beneficial in experimental asthma.

STIM/ORAI channels, responsible for store-operated Ca^{2+} entry in lymphocytes

In immune cells and especially lymphocytes, the most known calcium selective channels are the SOC ORAI1 calcium channels that are activated following ER Ca^{2+} store depletion [20–23]. Indeed the T-cell receptor (TCR) recognition of its ligand (antigen peptide presented by self MHC molecules) induces the recruitment and phosphorylation of substrates (enzymes and adapters) by tyrosine kinases, which results in activation of

Figure 2



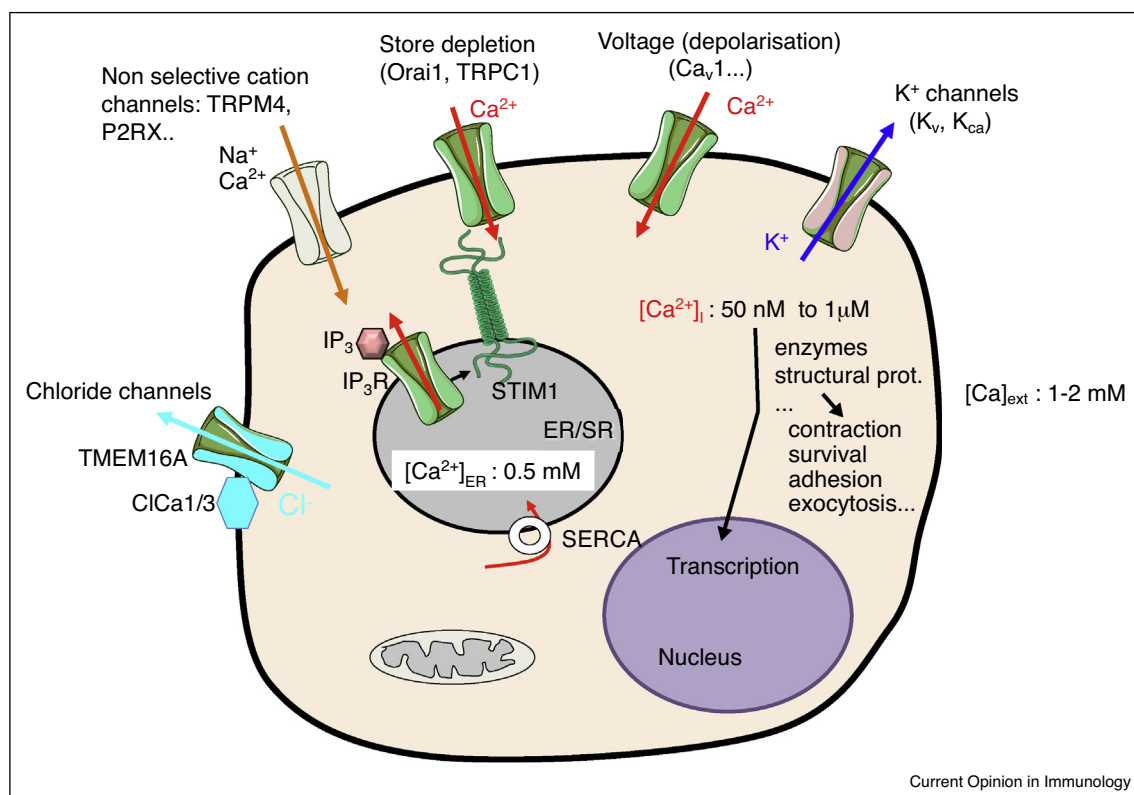
Involvement of IL-13 in the key manifestations of allergic asthma. Numerous cells in asthmatic lungs, including ILC2, Th2, mast cells, eosinophils, and some macrophages produced IL-13 (orange arrows). IL-13 induces the expression of V-CAM1 on endothelial cells promoting inflammatory cell recruitment in the lungs. It acts on epithelial cells that contribute to inflammation, fibrosis and goblet cell activation via the production of leukotrienes as LTC4. IL-13 increases the expression of chloride channel and associated proteins involved in mucus secretion. It promotes smooth muscle cell contraction by increasing the intracellular calcium concentration ($[Ca^{2+}]_i$) possibly through the production of the second messenger cADPR. IL-13 also inhibits NO-mediated smooth muscle relaxation. IL-13 might induce the proliferation (prolif.) of myofibroblasts, promote fibrosis through the production of TGFβ, of the metalloprotease MMP9 that induces active TGFβ, by the production of collagen from proline. Thus directly or not, IL-13 is involved in all the features of asthma: inflammation, airway remodeling, mucus secretion, airway hyper-reactivity (AHR) and fibrosis. LN: Lymph node.

downstream signaling pathways. Among them, phospholipase Cγ1 is activated, generating inositol 1,4,5 triphosphate (IP3) that binds to its receptor at the membrane of the ER, releasing Ca^{2+} into the cytosol. The depletion of ER Ca^{2+} stores changes the conformation of the ER Ca^{2+} sensor, STIM-1 resulting in its oligomerization and migration to come in touch with the ORAI1 calcium channel at the cell membrane (reviewed in Refs. [20–23]). This allows Ca^{2+} to enter from the external medium, to refuel the ER stores and to mediate cell activation. Synta 66 is considered as a selective inhibitor of ORAI channels and has some beneficial effect in an experimental model of asthma induced by OVA in the rat [24]. Brown-Norway rats that were immunized with OVA in alum and challenged with inhaled OVA were protected from asthma, which was associated with decreased

cytokine production, airway inflammation and airway hyper-reactivity. The ORAI channel is also at play in the response of epithelial cells to allergens. The allergen binds to protease activated receptor (PAR2), leading to an increase in intracellular calcium concentration ($[Ca^{2+}]_i$), IL-6 and TSLP production which are inhibited by the STIM/ORAI inhibitor BTP2 [25]. Although STIM/ORAI may appear as an interesting target in the treatment of allergy, this could result in global immunosuppression.

Voltage-gated (Ca_v)1 channels characterize excitable cells but are also important actors of Th2-cell functions
 Ca_v channels include Ca_v1 to Ca_v3 channels classified on sensitivity to drugs or venoms and on electrophysiological properties. Ca_v1 alias L-type calcium channels are defined as activated by strong cell depolarization and

Figure 3



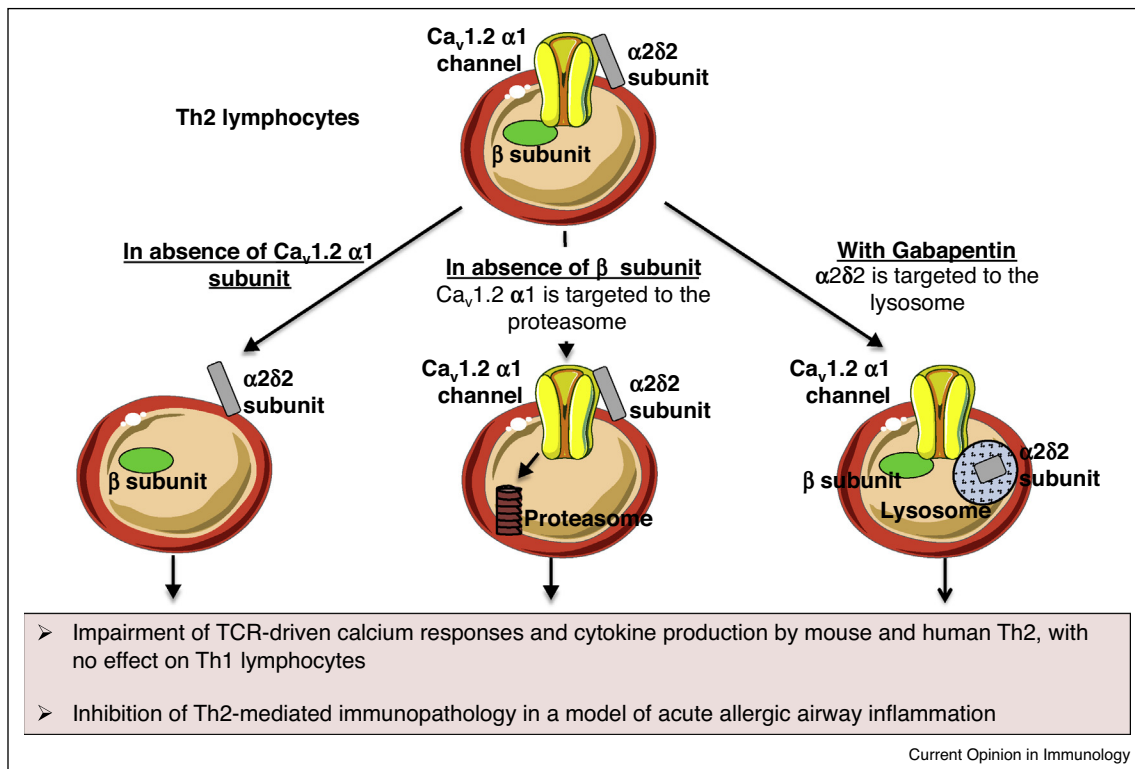
Ion channels as possible targets in allergic asthma. Ca^{2+} is a second messenger essential for most of cell functions. Its intracellular concentration ($[\text{Ca}^{2+}]_i$) is sharply regulated and some of the channels at the cell membrane appear as targets in allergy. The depletion of endoplasmic reticulum (ER) calcium stores induced by the binding of inositol triphosphate (IP_3) to its receptor (IP_3R) on the membrane of the ER unleashes Ca^{2+} from STIM-1 that oligomerize and come in contact with ORAI1 enabling its opening and Ca^{2+} entry (a phenomenon called store-operated Ca^{2+} entry: SOCE). STIM-1 might also activate TRPC1 , also inducing SOCE. Voltage-operated Ca^{2+} calcium (Ca_v) channels expressed by both excitable and nonexcitable cells) can be targets in allergy. Potassium channels (K_v or K_{ca}) cause an efflux of K^+ ions out of the cells and maintain the electrochemical driving force required for Ca^{2+} entry. Nonselective cation channels as TRPM4 or P2RX (activated mainly by ATP) play a role in the cell functions because they convey ions that control the cell membrane potential or Ca^{2+} itself. The Cl^- channels are important for mucus secretion.

play an important role in neurons, muscles, heart, and neuroendocrine tissues. Ca_v1 channels are formed the highly Ca^{2+} selective $\alpha1$ subunit that conveys the ions and auxiliary β and $\alpha2\delta$ subunits. Four genes (from $\text{Ca}_v1.1$ to $\text{Ca}_v1.4$) encode Ca_v1 $\alpha1$ subunits with some tissue-restricted pattern of expression. The dichotomy between nonexcitable and excitable cells where SOCC and VOCC would be at play respectively is no longer relevant. For example, several groups showed that Ca_v1 channels are expressed and functional in T and B-lymphocytes [26–31]. In this situation, the pattern of expression of Ca_v1 channels depends on the differentiation and activation status.

Thus $\text{Ca}_v1.4$ is expressed in naive T lymphocytes where they are important for the achievement of normal T-cell development and for Ca^{2+} signaling in naive T-cells [29]. Interestingly, upon activation the expression of these channels decreased [32]. Our group showed that

$\text{Ca}_v1.2$ and at a lesser degree $\text{Ca}_v1.3$ expression characterizes both mouse and human Th2-cells [32,33]. Th2-cells up-regulate CACNA1C mRNA (that encodes $\text{Ca}_v1.2$ $\alpha1$) late during Th2-cell differentiation, only after induction of GATA-3 [33], the master Th2 transcription factor. Ca_v1 channels have no obvious role in Th2-cell differentiation but they are required for proper Th2-cell functions. Indeed specific Ca_v1 blockade by nifedipine strongly diminishes TCR-driven calcium response and Th2-cytokine production [34]. These effects are recapitulated by transfecting Th2-cells with specific $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ antisense (AS) oligonucleotides [33]. Interestingly, the development and activation of Th1-cells is associated with extinction of $\text{Ca}_v1\alpha1$ expression [34] and neither nifedipine [34,35] nor Ca_v1AS [33] affect their functions. We tested their effect on Th2-mediated diseases as allergic asthma. As expected, nifedipine [34] or Ca_v1AS [33] have a strong beneficial effect in models of experimental asthma, without impact on Th1-mediated

Figure 4



All the subunits of Ca_v1 channels are required for Th2-lymphocyte function and acute allergic airway inflammation. We have shown by genetic and pharmacologic approaches that the knockdown of Ca_v1.2 α1 or β subunits or the α2δ2 inhibition by gabapentin decreases strongly the TCR-induced cytokine and calcium response in both mouse and human Th2 lymphocytes. Moreover, targeting of each component of the Ca_v1.2 calcium channel inhibits all cardinal parameter of mouse acute allergic airway inflammation without global immunosuppression.

diseases [33]. There is no evidence that these channels are voltage-operated in lymphocytes, raising the question of their regulation. In human Th2-cells, phorbol myristate acetate, an activator of classical and novel PKC induces Ca²⁺ entry, strongly reduced by nifedipine [32]. PKCα seems to be a good contributor to Ca_v1 regulation as PKCα blockade also diminishes the calcium response elicited by TCR stimulation [32] in human Th2-cells. Interestingly, Ca_v1.2 opening can become voltage-independent in smooth arteriolar muscle cells. This is an interesting issue because stretching of muscle fibers induces Ca_v1-dependent Ca²⁺ entry, which is not associated with strong cell membrane depolarization. Navedo and colleagues discovered that few Ca_v1.2 channels support an entry of Ca²⁺, independent of the cell membrane potential [36^{••},37,38] because they interact with PKCα. This interaction increases the open probability of Ca_v1.2 to 0.2, which is much higher than for isolated Ca_v1.2, even at depolarized cell membrane potential [36^{••}]. Auxiliary β and α2δ subunits are important for proper trafficking and functions of Ca_v1 and Ca_v2 channels in excitable cells [39]. We showed that human and mouse Th2-cells express mainly Ca_vβ1, β3, and α2δ2. Knocking-down Ca_vβ and α2δ blockade with

gabapentin, a drug used in the treatment of chronic neuropathies alter mouse and human Th2-functions and are beneficial in the treatment of experimental asthma [40].

Ca_vβ prevents Ca_v1.2α1 degradation by the proteasome in Th2-cells [40] as described in excitable cells [41,42] and gabapentin promotes the routing of α2δ2 to acidic compartments and its degradation in Th2-cells [40]. Therefore, auxiliary subunits appear also as important for the function of the channel at the cell membrane (Figure 4), leading us to propose that the channel comprising all the subunits is required for Th2-cell functions. Interestingly, gallopamil a Ca_v1 antagonist improved airway remodeling in severe asthmatic patients via its effect on airway smooth muscles [43].

An important issue concerns the role of Ca_v1 channels in shaping the calcium signature of Th2-cells in addition to the complex STIM/ORAI. STIM1 appears to be a hub between these two pathways because STIM1 can interact with Homer proteins [44] and Ca_v1.2 [45] in the case of ER Ca²⁺ depletion. This could attenuate Ca_v1.2 activity [45] favoring ORAI-1 dependent Ca²⁺ entry and refueling

the stores. Alternatively, it has been proposed that TRPC1, ORAI1, and $\text{Ca}_v1.2$ can form a signal complex in vascular smooth muscle cells concurring to Ca^{2+} entry in these cells. Therefore, it will be important to investigate the contribution of STIM/ORAI and Ca_v1 pathways in Th2-cells.

Potassium channels, regulators of the calcium influx can be targeted in asthma

These channels extrude the potassium from the cytosol toward the external medium along their chemical gradient and tend to hyperpolarize the cell membrane. Their activation is required to maintain the electrochemical driving force allowing Ca^{2+} entry. More than 90 genes encode the main subunit of potassium channels in humans (reviewed in Ref. [46]). K^+ channels include K_v and K_{Ca} channels that are activated by cell membrane depolarization and by an increase in $[\text{Ca}^{2+}]_i$, respectively. $\text{K}_{Ca3.1}$ and $\text{K}_v1.3$ are found in T-lymphocytes and their relative expression depends upon the type of T-cells (naïve, effector memory, and central memory). $\text{K}_{Ca3.1}$ (expressed in T and B lymphocytes, mast cells, and bronchial smooth muscle cells) deletion is beneficial in a model of asthma induced by OVA [47]. $\text{K}_v1.3$ are expressed by number of T-cells infiltrating the lower airways of asthmatic patients and a selective inhibitor reduces their Th2 cytokine production and is effective in an experimental of asthma induced by OVA [48,49].

Nonselective cation channels also participate to the activation of inflammatory cells and asthmatic reactions

They encompass TRP channels (comprising TRPC, TRPV, TRPA, and TRPM family members) or purinergic P2X receptors. Extracellular ATP activates these latter receptors. Nonselective cation channels can interfere with cell activation because, they convey cations and therefore modify the cell membrane potential or they can support Ca^{2+} entry or contribute to Ca^{2+} influx into T-cells.

TRPM4, a nonselective calcium channel, implicated in tuning the cell membrane potential and thus imposing an oscillatory Ca^{2+} pattern

Thus T lymphocytes express TRPM4 channels that mainly conduces Na^+ ions and are activated following Ca^{2+} entry. T-cell activation induces Ca^{2+} entry that activates TRPM4 responsible for Na^+ influx into the cells, which further contributes to cell membrane depolarization, braking calcium influx due to a reduction in the electrochemical force driving Ca^{2+} . TCR-driven increase in $[\text{Ca}^{2+}]_i$ and/or cell membrane depolarization activates K_{Ca} and/or K_v channels respectively, leading to K^+ efflux which tends to hyperpolarize the cell membrane and favors Ca^{2+} influx with a subsequent new activation of TRPM4 and so on. Therefore the absence of TRPM4 tends to suppress the oscillatory pattern in T-cells [50].

Th2 cells express three times more TRPM4 than Th1-cells [51]. These authors investigated the effect of TRPM4 knock down in both Th1 and Th2-cells. In the absence of TRPM4, the peak of Ca^{2+} is higher with more oscillations in Th2-cells. This is associated with higher nuclear NFAT localization upon activation. Th2-cells with reduced expression of TRPM4 are more motile, produce more IL-2 but less IL-4 [51]. Surprisingly, the inhibition of TRPM4 in Th1-cells have opposite effects compared to Th2-cells (decreased Ca^{2+} signal, NFAT nuclear localization, mobility and IFN γ production) [51]. These results suggest different regulation of the Ca^{2+} response by TRPM4 in Th1 and Th2-cells but the mechanisms are still unknown.

Noncationic channels as TRPC may modulate Ca^{2+} influx in T-cells

TRPC1 and TRPC3 channels are expressed in T-cells. Their activations are phospholipase C and diacylglycerol-dependent respectively. It has been suggested that TRPC1 may interact with STIM-1. TRPC1 can mediate a Ca^{2+} influx and could also interfere with ORAI-dependent calcium entry even if the role of TRPC1 is not so clear [52,53]. $\text{TRPC1}^{-/-}$ mice are less susceptible to OVA-induced asthma [54] suggesting an implication of this channel in allergy. The decreased ability of splenic cells from $\text{TRPC1}^{-/-}$ to produce cytokines including Th2-cytokines upon stimulation with the antigen suggest that TRPC1 is involved in the priming of allergen-specific Th2-cells.

Purinergic receptors can induce an influx of Ca^{2+} following stimulation by external ATP

They can mediate an influx of Ca^{2+} as shown for P2RX receptors and especially P2RX4 and P2RX7 that are expressed by DCs, macrophages, mast cells, T-cells, and epithelial cells. ATP released by damaged tissue activates immune cells sustaining inflammation and airway hyper-responsiveness as shown by its ability to promote histamine release by human mast cells in a P2RX7 dependent manner [55]. In addition, mice with genetic ablation of P2RX7 [56] or P2RX4 [57] are protected from the development of OVA-induced asthma.

Chloride channels

Secretion of chloride through chloride channels controls the quality and the quantity of mucus that lines the airways. An overproduction of mucus is found in asthmatic patients and IL-13 a key type 2 cytokine promotes the expression of chloride channel and of mucins, as well as goblet-cell metaplasia. The chloride channel is now identified as TMEM16A [58], and CLCA1 (for calcium-dependent chloride channel associated protein) is a secreted protein that associates with TMEM16A and modulates its stability at the cell membrane [59]. Interestingly, IL-4 and IL-3 up-regulate both TMEM16A and CLCA1 in *in vitro* culture of bronchial epithelial cells.

TMEM16A inhibitors decrease mucus secretion and alleviate airway hyper-reactivity in a model of asthma [60].

Because of the number of pathways involved in asthma and to the differential expression of ion channels on the cell types considered. $\text{Ca}_v1.2$ channels are of special interest since their blockade can interrupt Th2-mediated inflammation and such blockers could also impact airway hyper-reactivity through their action on smooth muscle bronchiolar cells.

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Conflict of interest statement

None declared.

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