The β and α2δ auxiliary subunits of voltage-gated calcium channel 1 (Ca_v1) are required for T_H2 lymphocyte function and acute allergic airway inflammation

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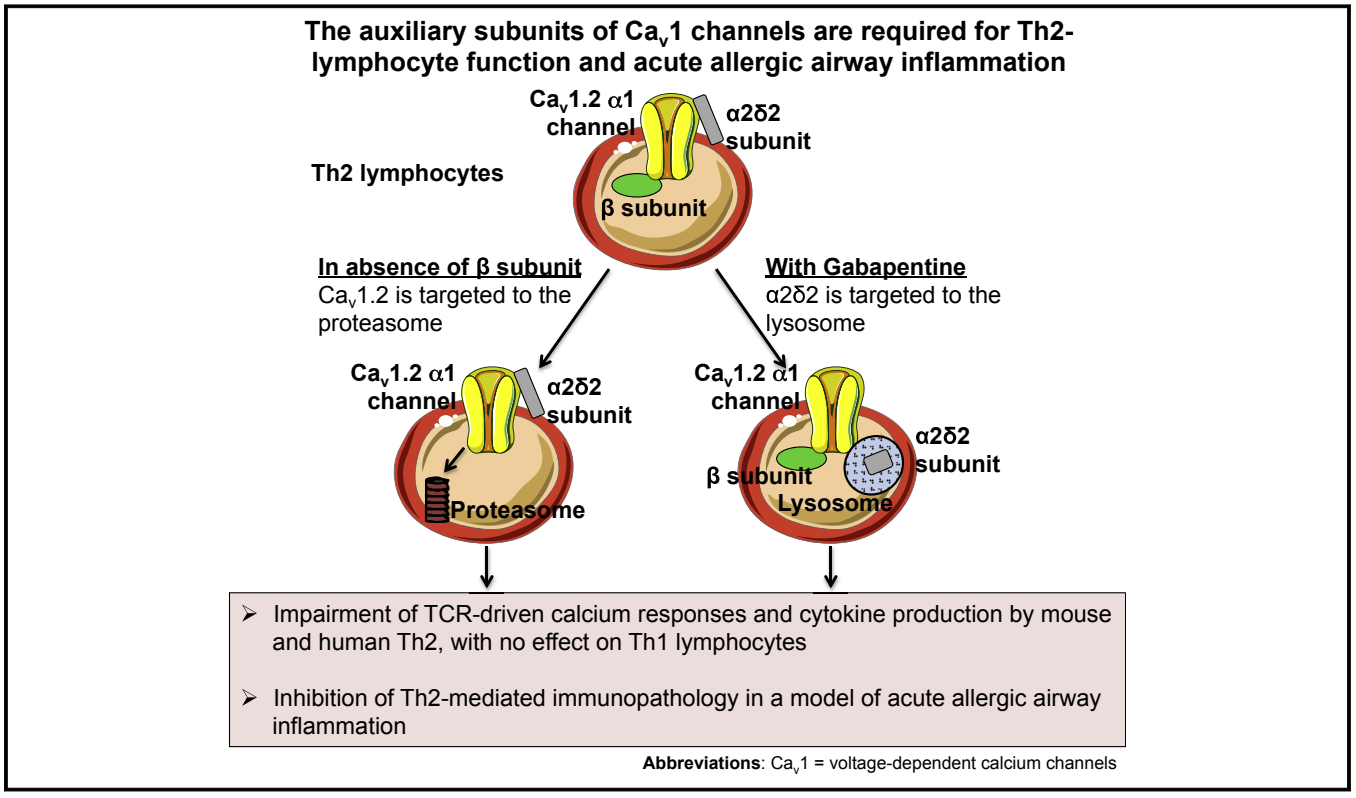
GRAPHICAL ABSTRACT



Background: T lymphocytes express not only cell membrane ORAI calcium release–activated calcium modulator 1 but also voltage-gated calcium channel (Ca_v) 1 channels. In excitable cells these channels are composed of the ion-forming pore α1 and auxiliary subunits (β and α2δ) needed for proper trafficking and activation of the channel. Previously, we disclosed the role of $Ca_v1.2$ α1 in mouse and human T_H2 but not T_H1 cell functions and showed that knocking down Ca_v1 α1 prevents experimental asthma.

Objective: We investigated the role of β and α2δ auxiliary subunits on Ca_v1 α1 function in T_H2 lymphocytes and on the development of acute allergic airway inflammation.

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Methods: We used $Ca_v\beta$ antisense oligonucleotides to knock down $Ca_v\beta$ and gabapentin, a drug that binds to and inhibits $\alpha2\delta1$ and $\alpha2\delta2$, to test their effects on T_H2 functions and their capacity to reduce allergic airway inflammation.

Results: Mouse and human T_H2 cells express mainly $Ca_v\beta1$, $\beta3$, and $\alpha2\delta2$ subunits. $Ca_v\beta$ antisense reduces T-cell receptor–driven calcium responses and cytokine production by mouse and human T_H2 cells with no effect on T_H1 cells. $Ca_v\beta$ is mainly involved in restraining $Ca_v1.2$ $\alpha1$ degradation through the proteasome because a proteasome inhibitor partially restores the $\alpha1$ protein level. Gabapentin impairs the T-cell receptor–driven calcium response and cytokine production associated with the loss of $\alpha2\delta2$ protein in T_H2 cells.

Conclusions: These results stress the role of $Ca_v\beta$ and $\alpha2\delta2$ auxiliary subunits in the stability and activation of $Ca_v1.2$ channels in T_H2 lymphocytes both *in vitro* and *in vivo*, as demonstrated by the beneficial effect of $Ca_v\beta$ antisense and gabapentin in allergic airway inflammation. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Asthma, T_H2, voltage-gated calcium channel 1, calcium, cytokines*

Abbreviations used

BAL:	Bronchoalveolar lavage
$[Ca^{2+}]_i$:	Intracellular Ca^{2+} concentration
Ca_v:	Voltage-gated calcium channel
$Ca_v\beta$AS:	$Ca_v\beta$ antisense
$Ca_v\beta$S:	$Ca_v\beta$ scrambled
CRTH2:	Chemoattractant receptor–homologous molecule expressed on T_H2 cells
ER:	Endoplasmic reticulum
HPRT:	Hypoxanthine-guanine phosphoribosyltransferase
ORAI1:	ORAI calcium release–activated calcium modulator 1
OVA:	Ovalbumin
TCR:	T-cell receptor

Allergic diseases, including rhinitis, atopic dermatitis, asthma, and food allergies, are induced by T_H2 lymphocytes. T_H2-type responses are characterized by production of IL-4, IL-5, and IL-13, which contribute to mucus production, eosinophilia, and high levels of antigen-specific IgE. At present, treatments for allergic asthma are often symptomatic, even if in some cases specific allergenic immunotherapy and treatments targeting the cytokines (or their receptors) involved in type 2 inflammation can be beneficial.[1,2] The main immunosuppressants (eg, cyclosporine and tacrolimus) have also been proposed for use in patients with severe asthma resistant to glucocorticoids. However, because they decrease the activity of T cells and therefore the overall immune response by acting on calcium signaling, they have adverse effects.

Calcium is a second messenger that plays specific and key roles in various cellular functions, such as activation, differentiation, proliferation, and death. The role of store-operated Ca^{2+} entry is well described, implicating the sensing of T-cell receptor (TCR)–driven endoplasmic reticulum (ER) Ca^{2+} depletion by stromal interaction molecule 1, its oligomerization, and its localization in the vicinity of calcium release–activated calcium modulator 1 (ORAI1) channels at the plasma membrane, permitting sustained Ca^{2+} entry.[3]

In addition to these channels, the role of voltage-gated calcium channel (Ca_v) 1 channels (defined as voltage activated in excitable cells) in T lymphocytes is now accepted.[4-11]

In excitable cells, Ca_v1 channels are composed of the ion-forming pore $\alpha1$ and auxiliary β and $\alpha2\delta$ subunits, with each subunit being encoded by 4 genes. $Ca_v1.1$ to $Ca_v1.4$ $\alpha1$ form the ion pore and support the biophysical and pharmacologic properties of the channel,[12-14] whereas auxiliary β and $\alpha2\delta$ subunits increase Ca_v currents by enhancing the number of channels at the cell membrane and favoring channel opening.[15-19] $Ca_v\beta$ would act by facilitating the correct folding of $\alpha1$ and promoting its exit from the ER,[20] whereas $Ca_v\alpha2\delta$ increases insertion of the channel into the cell membrane by favoring the trafficking of the channel from the post-Golgi apparatus and decreasing its turnover.[21-23]

While $Ca_v\beta2$ deletion[24] inhibited thymocyte development, $Ca_v\beta3$ and $Ca_v\beta4$ were found to be important for calcium influx, nuclear factor of activated T cells (NFAT) nuclear translocation, and cytokine production by peripheral $CD4^+$ T lymphocytes. Previously, we reported that $Ca_v1.2$ was expressed and functional in human and mouse T_H2 cells.[7,9,25] In contrast, T_H1 and T_H17 lymphocytes in both species lacked $Ca_v1.2$ expression.

Here we address whether Ca_v auxiliary subunits are required for channel functions in T_H2 lymphocytes. We show that $Ca_v\beta1$, $Ca_v\beta3$, and $\alpha2\delta2$ subunits are expressed in mouse and human T_H2 cells. Knocking down $Ca_v\beta$ promoted degradation of $Ca_v1.2$ $\alpha1$, which was at least partly rescued by adding MG132, a proteasome inhibitor, whereas gabapentin, an inhibitor of $\alpha2\delta1/2$ subunits, decreased $\alpha2\delta2$ protein levels in T_H2 lymphocytes. In both cases, it was associated with a decreased TCR-dependent intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase and T_H2 cytokine production. In accordance with our *in vitro* results, targeting either $Ca_v\beta$ or $\alpha2\delta$ *in vivo* was beneficial in a model of acute allergic airway inflammation.

METHODS

More details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Mice and model of acute airway allergic inflammation

Eight- to 12-week-old female BALB/c mice were obtained from Janvier (Le Genest St Isle, France), and TCR ovalbumin (OVA) transgenic DO11.10 mice were maintained in our pathogen-free animal facility. The INSERM U1043 Institutional Review Board for animal experimentation approved protocols. BALB/c mice immunized intraperitoneally with OVA (100 μg) in alum (2 mg) were 15 days later administered intranasal OVA (50 μg/d) in PBS for 5 days, as previously described,[7] with or without $Ca_v\beta$ scrambled ($Ca_v\beta$S) or $Ca_v\beta$ antisense ($Ca_v\beta$AS) oligonucleotides (200 μg/d) or gabapentin (400 mg/L in drinking water) that was renewed every other day.[26] For T_H2 transfer experiments, BALB/c mice (Janvier) were transferred intravenously with 3×10^6 D011.10 T_H2 cells transfected with $Ca_v\beta$S or $Ca_v\beta$AS and given intranasal OVA (50 μg/d) for 5 days. Twenty-four hours after the final OVA administration, serum, bronchoalveolar lavage (BAL) fluid, lungs, and draining lymph nodes were collected and processed, as described in the Methods section in this article's Online Repository.

Cell culture

Mouse T_H1 and T_H2 cells were generated by weekly stimulation of DO11.10 $CD4^+$ T cells with antigen-presenting cells and the 323-339 OVA peptide plus appropriate differentiation cocktails: IL-12 (5 ng/mL) and anti–IL-4 antibody (11B11, 10 μg/mL) for T_H1 and IL-4 (5 ng/mL) and