Physiological significance of delayed rectifier $K^+$ channels ($Kv1.3$) expressed in T lymphocytes and their pathological significance in chronic kidney disease

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Abstract  T lymphocytes predominantly express delayed rectifier $K^+$ channels ($Kv1.3$) in their plasma membranes. More than 30 years ago, patch-clamp studies revealed that the channels play crucial roles in facilitating the calcium influx necessary to trigger lymphocyte activation and proliferation. In addition to selective channel inhibitors that have been developed, we recently showed physiological evidence that drugs such as nonsteroidal anti-inflammatory drugs, antibiotics, and anti-hypertensives effectively suppress the channel currents in lymphocytes, and thus exert immunosuppressive effects. Using experimental animal models, previous studies revealed the pathological relevance between the expression of ion channels and the progression of renal diseases. As an extension, we recently demonstrated that the overexpression of lymphocyte $Kv1.3$ channels contributed to the progression of chronic kidney disease (CKD) by promoting cellular proliferation and interstitial fibrosis. Together with our in-vitro results, the studies indicated the therapeutic potency of $Kv1.3$-channel inhibitors in the treatment or the prevention of CKD.

Keywords  Lymphocytes · Delayed rectifier $K^+$ channels ($Kv1.3$) · Membrane capacitance ($C_m$) · Immunomodulatory effects · Overexpression of $Kv1.3$ channels · Chronic kidney disease (CKD)

Introduction

Thymus-derived lymphocytes (T lymphocytes) predominantly express delayed rectifier $K^+$ channels ($Kv1.3$) in their plasma membranes [1–3]. Patch-clamp studies revealed that the channels generate the $K^+$-diffusion potential across the plasma membranes, and thus play roles in regulating resting membrane potential and controlling the cell volume [4, 5]. Using selective channel inhibitors, later studies have further demonstrated that the $Kv1.3$ channels also play crucial roles in facilitating the calcium influx necessary to trigger lymphocyte activation and proliferation [3, 6, 7]. Concerning the molecular mechanisms that are involved, the rise in the intracellular calcium concentration activates phosphatase calcineurin, which then dephosphorylates the nuclear factor of activated T cells (NFAT), enabling it to accumulate in the nucleus and bind to the promoter of the gene encoding interleukin 2 (IL-2) [8] (Fig. 1). So far, pharmacological targeting of calcineurin has been regarded as the main mechanism by which immunosuppressive reagents, such as cyclosporine and tacrolimus (FK506), exert their effects [9]. Additionally, recent advances in functional pharmacology revealed that a selective inhibition of lymphocyte $Kv1.3$ channels also represses lymphocyte activity and thus suppresses cellular immunity [10] (Fig. 1). Nonsteroidal anti-inflammatory drugs (NSAIDs), macrolide antibiotics, and $Ca^{2+}$ channel blockers (CCBs) are known to exert immunomodulatory properties besides their respective anti-inflammatory, anti-microbial, and anti-hypertensive effects [11–16]. Since these drugs are lipophilic [17–20], they would directly disturb the lymphocyte plasma membranes, and thus affect the $Kv1.3$ channel currents when they modulate the immune response.

“Chronic inflammatory diseases” is a category of diseases for which “chronic inflammation” or “the over-stimulation of cellular immunity” is responsible for pathogenesis [21]. In addition to infectious diseases and autoimmune disorders, a number of diseases such as
cancer, neuroinflammatory diseases, and metabolic disorders nowadays fall into this category [22] (Fig. 2). Chronic kidney disease (CKD) progresses relentlessly to end-stage renal disease (ESRD). The histopathology of kidneys in ESRD is characterized by tubulointerstitial fibrosis in both humans [23, 24] and experimental animal models [25–27]. Previous studies revealed the initial involvement of inflammatory leukocytes, such as T lymphocytes, macrophages, and mast cells, in the development of renal fibrosis [28, 29]. Therefore, CKD is nowadays regarded as one of the “chronic inflammatory diseases” [30] (Fig. 2). Since lymphocytes are activated [31] and serum cytokine levels are greatly elevated in patients with ESRD [32, 33], the Kv1.3 channels expressed in lymphocytes would contribute to the progression of renal fibrosis in advanced CKD.

In the first part of this review article, I summarize the results of in-vitro patch-clamp studies that demonstrated that NSAIDs, macrolide antibiotics, and CCBs suppress thymocyte Kv1.3 channel currents and the membrane capacitance (Cm). Then, in the second part, I summarize the results of in-vivo animal studies that revealed the histopathological features of the kidneys from advanced chronic renal failure (CRF) and demonstrated the involvement of Kv1.3-channel expression in the pathogenesis. This review provides an overview of the physiological and pathological significance of the Kv1.3 channels expressed in T lymphocytes. It also provides a speculation on the key roles of the channels in the pathogenesis and treatment of CKD.

**Physiological significance of Kv1.3 channels in T lymphocytes**

**Electrophysiological properties of Kv1.3 channels in T lymphocytes**

T lymphocytes express a variety of ion channels in their plasma membranes, such as K⁺ channels, Ca²⁺ channels, and Cl⁻ channels [34]. The major types of these channels that have extensively been studied include voltage-dependent K⁺ channels (Kv), Ca²⁺-activated K⁺ channels (KCa), Ca²⁺ release-activated Ca²⁺ channels (CRAC), Mg²⁺-inhibited Ca²⁺-permeable current (MIC) channels, and swelling-activated Cl⁻ channels (Clswell) [35–39]. Among them, Cahalan et al. [1, 2] initially identified in their patch-clamp studies that human T lymphocytes most predominantly express Kv1.3-channels in their plasma membranes. In our recent patch-clamp studies, stepwise changes in the membrane potential, from the holding potential of −80 mV to the various depolarizing potential levels, evoked membrane currents in thymus-derived murine lymphocytes (thymocytes), showing voltage-dependent activation and inactivation patterns characteristic to Kv1.3 [40–43]. Additionally, since the currents were almost totally abolished by margatoxin, a selective inhibitor of Kv1.3 channels [40], they were identified as Kv1.3-channel currents.

Previous patch-clamp studies revealed that Kv1.3 channels in lymphocytes generate the K⁺-diffusion potential across the plasma membranes, and thus play roles in regulating the resting membrane potential and controlling the cell volume [4, 5]. By generating a driving force for the calcium influx, the membrane hyperpolarization brought about by the opening of Kv1.3 channels also stimulates the calcium signaling necessary to trigger lymphocyte activation and proliferation [6, 7, 34]. Through the modification of the chemical structures of venom, scorpion, or sea anemone peptide toxins, highly selective inhibitors
of the channel have been engineered [44–47]. Recently, their immunomodulatory properties were actually demonstrated in the treatment of autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, and type 1 diabetes mellitus [45, 48, 49]. In these disorders, the inhibition of the Kv1.3 channel modulated calcium influx patterns in T lymphocytes, and thus exerted immunosuppressive effects [48].

Immunomodulatory properties of NSAIDs, macrolide antibiotics, and CCBs

NSAIDs are used in the treatment of autoimmune disorders, such as rheumatoid arthritis [50], systemic lupus erythematosus [51], and virus-triggered hypocomplementemia [52]. Studies revealed that NSAIDs inhibit the migration of leukocytes or their cytokine production either triggered by [53] or independently of [54, 55] cyclooxygenase (COX), and thus exert immunomodulatory effects. Recently, Villalonga et al. [56] have found electrophysiological relevance in this context, namely that diclofenac suppressed Kv1.3-channel currents in macrophages, and thereby impaired the migration of those cells.

Macrolide antibiotics are widely used for the treatment of a variety of bacterial infections [57]. Recently, their therapeutic efficacies as immunosuppressive agents have also been demonstrated in patients with diffuse panbronchiolitis [58], cystic fibrosis, and inflammatory bowel diseases [59]. According to several in-vitro studies, macrolide antibiotics, such as clarithromycin and azithromycin, suppress the production of pro-inflammatory cytokines from leukocytes and thus exert immunomodulatory effects [12–14]. Of all macrolides, clarithromycin most potently suppresses the production of IL-2 [56, 60], leukocyte migration assay [56], and whole-cell membrane capacitance [56, 60].

CCBs have been demonstrated to exert immunosuppressive properties in humans [15, 16], in addition to their cardiovascular effects in hypertension and ischemic heart disease [61]. According to several in-vitro studies, CCBs, including nifedipine, verapamil, and diltiazem, repress the migration of leukocytes or inhibit their proliferation [62, 63]. Recently, using human peripheral leukocytes, Matsumori et al. [64, 65] further demonstrated that nifedipine and verapamil also suppress the production of pro-inflammatory cytokines, such as IL-1β, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ).

Suppressive effects of NSAIDs and macrolide antibiotics on Kv1.3-channel currents in murine thymocytes

Previous patch-clamp studies, including ours, have revealed the physiological mechanisms by which NSAIDs and macrolide antibiotics exert immunomodulatory properties [41, 42]. In these studies, drugs such as diclofenac sodium (0.1 mM), salicylate (0.3 mM), indomethacin (0.1 mM), and clarithromycin (30, 100 μM) were applied to murine thymocytes, and the changes in the whole-cell membrane currents were examined. Table 1 summarizes the effects of these drugs on the peak and the pulse-end currents. As previously demonstrated in macrophages [56], diclofenac sodium and salicylate markedly suppressed the Kv1.3-channel currents in thymocytes [41]. However, differing from those in macrophages, the peak outward currents were not affected in thymocytes (Table 1). Instead, these drugs significantly suppressed the pulse-end currents. Indomethacin, a more potent anti-inflammatory drug than the others, both lowered the peak and the pulse-end currents (Table 1). Clarithromycin also suppressed Kv1.3-channel currents in thymocytes [42], as previously demonstrated with voltage-dependent K⁺-channel currents in cardiomyocytes [66, 67]. A dose of 100 μM of this drug lowered the pulse-end currents to the same extent as 30 μM (Table 1). However, it suppressed the peak currents more markedly than 30 μM, indicating the dose-dependent attenuating effects on the activation curves [66].

Since lymphocyte Kv1.3 channels trigger calcium influx, which is necessary for IL-2 synthesis [8], and since a channel blockade by highly selective inhibitors, including margatoxin and ShK-Dap²², actually repressed the immune response in lymphocytes [56, 68], the suppressive effects of NSAIDs and clarithromycin on the channel currents were thought to contribute to their immunomodulatory properties (Fig. 3). To determine the effects of the drugs on the lymphocyte activation kinetics, more detailed in-vitro functional analyses will be required. Possible approaches in the future would include the measurement of cytokine production [60], leukocyte migration assay [56], and the measurement of [³H] thymidine incorporation into the lymphocyte DNA [68].

Effects of CCBs on Kv1.3-channel currents and whole-cell membrane capacitance

**CCBs markedly inhibit Kv1.3-channel currents in murine thymocytes**

1,4-Dihydropyridine (DHP) CCBs, which are highly lipophilic compared to the other types of CCBs [69, 70], exert relatively stronger immunomodulatory effects [64, 65]. Among them, benidipine is one of the most lipophilic and the longest-acting [71, 72], and nifedipine is the prototype of DHPs [73]. In our patch-clamp study, nifedipine and benidipine both markedly suppressed the
Kv1.3-channel currents in murine thymocytes at doses as low as 100 and 10 μM, respectively (Fig. 4A) [43]. Of note, benidipine almost totally inhibited the pulse-end currents irreversibly (Fig. 4Ab).

Although the effects of CCBs on cytokine production have not been directly examined [64, 65], the marked inhibition of the channel currents by these drugs strongly suggests their higher immunosuppressive potency than that of NSAIDs or macrolide antibiotics [41, 42]. Moreover, the persistent effect of benidipine on the decreased channel currents may predict its longer duration of action in thymocytes, as previously demonstrated in cardiomyocytes [72] and isolated coronary arteries [74]. Verapamil, a phenylalkylamine CCB, also suppresses Kv1.3-channel currents in various cells [75–77]. Since verapamil facilitates the inactivation of the channel currents, the drug is considered to plug into the open pores of the channel to inhibit the currents [77]. In contrast, nifedipine or benidipine induced the current inactivation on a much slower time scale than verapamil did (Fig. 4A). This represents a “C-type inactivation” pattern in kinetic studies [78], suggesting that these drugs induced conformational collapse of

Table 1 Summary of changes in peak currents and pulse-end currents after application of NSAIDs and macrolide antibiotics

| NSAIDs          | N  | Peak current (%) | Pulse-end current/peak current (% | Before | After |
|-----------------|----|------------------|----------------------------------|--------|-------|
|                 |    |                  |                                  |        |       |
| 0.1 mM Diclofenac Na | 5  | 94.5 ± 4.3       | 17.0 ± 1.9 #                    |        |       |
| 0.3 mM Salicylate      | 5  | 89.8 ± 3.2       | 20.8 ± 2.9 #                    |        |       |
| 0.1 mM Indomethacin | 5  | 75.9 ± 4.8 #     | 29.3 ± 1.7 #                    |        |       |
| NSAIDs          | N  | Peak current density (pA/pF) | Pulse-end current/peak current (%) | Before | After |
|                 |    |                  |                                  |        |       |
| 30 μM Clarithromycin | 5  | 178 ± 5.6        | 47.5 ± 2.2                      |        |       |
| 100 μM Clarithromycin | 5  | 277 ± 4.4        | 48.5 ± 1.4                      |        |       |

Proposed mechanism of immunosuppression by nonsteroidal anti-inflammatory drugs and macrolide antibiotics. NSAIDs nonsteroidal anti-inflammatory drugs

Immunosuppression

Fig. 3 Proposed mechanism of immunosuppression by nonsteroidal anti-inflammatory drugs and macrolide antibiotics. NSAIDs nonsteroidal anti-inflammatory drugs

Fig. 4 Effects of Ca²⁺ channel blockers on Kv1.3-channel currents and whole-cell membrane capacitance in murine thymocytes. A The effects of 100 μM nifedipine (a) and 10 μM benidipine (b) on Kv1.3-channel currents. Typical whole-cell current traces at different voltage-steps recorded before and after either drug application, and after the washout. The currents were elicited by voltage-steps from the holding potential of −80 to 0, 40, and 80 mV, as depicted in the voltage protocol. Each pulse was applied for a 200-ms duration between 10-s intervals. B Benidipine-induced changes in the thymocyte membrane capacitance, series, and membrane conductance. After establishing the whole-cell configuration, the external solution containing 10 μM benidipine was delivered for 30 s to single thymocytes. Membrane capacitance, series, and membrane conductance were monitored for at least 2 min. N = 5 for each trace. Cm membrane capacitance, Gs series conductance, Gm membrane conductance. Modified from Ref. [43]
the selectivity filters (inactivation gates) within pore-forming domains of the K⁺ channels [40].

Benidipine irreversibly decreases the whole-cell membrane capacitance in murine thymocytes

Since DHP CCBs are known to interact with lipid bilayers of the plasma membranes [19, 20, 70], and since benidipine likely induces conformational collapse of the channels (Fig. 4A), it was conceivable that these drugs would generate structural changes in the thymocyte membranes. Microscopic changes in the cell surface area have best been monitored by the measurement of the whole-cell membrane capacitance (Cm) during exocytosis from secretory cells [79, 80] or thrombopoiesis from megakaryocytes [81–83]. Therefore, we employed this electrophysiological approach to detect the microscopic changes in the thymocyte membrane surface before and after the drug application [43]. Benidipine induced a significant decrease in Cm immediately after the application (Fig. 4B), with minimal changes in membrane conductance (Gm) and series conductance (Gs), indicating that this drug actually induced structural changes in the thymocyte membranes. The irreversible effect of benidipine on Cm suggested that the drug induced long-lasting structural changes in the thymocyte membranes.

The whole-cell Cm is calculated from a parallel-plate capacitor formula: Cm = εA/d, where ε is the dielectric modulus of the plasma membrane, A is the membrane surface area, and d is the membrane thickness [84]. Assuming that ε and A are relatively constant before and after the drug application, the increase in d is primarily considered to be responsible for the decrease in Cm [85] (Fig. 5A). Since benidipine is highly lipophilic [19, 20, 70], it would disperse easily into the lipid bilayers of the plasma membrane. Therefore, the decrease in the Cm (Fig. 4B) was thought to represent increased membrane thickness (d) as a result of the accumulation of benidipine in the plasma membrane (Fig. 5B). The accumulated drug may directly perturb the composite domains of the channels from inside the membranes (Fig. 5B). This would include the constriction or the conformational collapse of the selectivity filters within the pore-forming domains [78]. Thus, benidipine was thought to induce the “C-type inactivation” patterns of the channel currents (Fig. 4A). Benidipine, which has a higher partition coefficient than any other DHP CCBs [69, 86], would associate more strongly and dissociate more slowly with the DHP receptor binding sites in the membranes [19, 20, 70]. Therefore, benidipine was thought to accumulate in the membranes for a long period of time, causing the persistent blockade of the channel currents (Fig. 4Ab) and the long-lasting decrease in the Cm (Fig. 4B).

Pathological significance of Kv1.3 channels in the progression of CKD

Involvement of lymphocyte Kv1.3 channels in the pathogenesis of renal diseases

In patients with ESRD, peripheral lymphocytes are activated [31] and serum cytokine levels are known to be elevated [32, 33]. A patch-clamp study found an electrophysiological relevance in this context, demonstrating increased conductance of voltage-dependent K⁺ channels in lymphocytes of these patients [87]. The study also revealed that the activity of the channels was deeply associated with the severity of CRF. In experimental animal models with renal diseases, such as kidney allograft rejection [88] and glomerulonephritis [89], immunosuppression by the blockade of lymphocyte Kv1.3 channels actually prevented or ameliorated the progression of the diseases. The studies demonstrated the involvement of the channels in the pathogenesis of renal diseases, for which “chronic inflammation” or “the overstimulation of cellular immunity” is responsible.

In-situ proliferation of inflammatory leukocytes in kidneys with advanced CKD

So far, several laboratory models of CRF have been described, such as the diminution of renal mass by surgery [90, 91], ligation of renal artery branches [92], toxic nephritis [93, 94], and immunologically-induced nephritis [95, 96]. Among them, rat models with 5/6 nephrectomy (subtotal nephrectomy) followed by 8–10-week recovery periods have most popularly been studied, since they are less lethal and thus represent the stable and far advanced renal failure [97–99]. Histologically, the rat kidneys with
subtotal nephrectomy are mainly characterized by severe glomerulosclerosis as a result of increased glomerular pressure and protein overload [25, 26]. In advanced stages, however, these kidneys are additionally characterized by diffuse interstitial fibrosis [27, 29], which was originally reported by Kumano et al. [28] as showing the involvement of leukocyte infiltration. In our study, rats with 5/6 nephrectomy followed by longer recovery periods were used as the model of advanced CRF [100]. In these rat kidneys, in addition to diffuse fibrosis [25, 27, 29], a large number of inflammatory leukocytes such as T lymphocytes and macrophages infiltrated into the cortical interstitium (Fig. 6Ab vs. a) with significant increases in their marker expression (Fig. 6Ac). The immunohistochemistry for Ki-67 demonstrated a number of positively stained, small round cells (Fig. 6Bb vs. a), indicating that inflammatory leukocytes were proliferating within the cortical interstitium of advanced CRF rat kidneys.

In the development of tubulointerstitial fibrosis in CRF rat kidneys, inflammatory leukocytes are initially recruited from the bone marrow and infiltrate into the renal interstitium to trigger the proliferation of fibroblasts [101]. Then, with the progression of uremia, the number of such leukocytes is considered to decrease due to the decrease in circulating lymphocyte counts [102]. In advanced CRF, however, our study demonstrated that the numbers of leukocytes in the cortical interstitium were dramatically increased by in-situ proliferation [100], showing pathological features similar to those of acute glomerulonephritis [103]. Since the cytokines produced by leukocytes stimulate the activity of fibroblasts to produce collagen [101], the increased number of leukocytes in the interstitium would promote the progression of renal fibrosis and thus contribute to the rapid deterioration of renal function in advanced CRF [100].

Overexpression of Kv1.3 channels in the leukocytes of advanced CRF kidneys

Lymphocytes and macrophages express Kv1.3 channels in their plasma membranes [3, 56]. The membrane hyperpolarization brought about by the opening of the channels facilitates the calcium influx necessary to trigger
lymphocyte activation [6, 7, 34]. In normal rat kidneys, Kv1.3 channels are expressed in some proximal tubules [89], and predominantly in the basolateral membranes of the inner medullary collecting duct cells [104]. In these cells, the channels may contribute to the maintenance of the driving force for Na\(^+\) reabsorption or play roles in cellular or total body fluid volume regulation. In advanced CRF rat kidneys, however, Kv1.3 channels were overexpressed in the proliferating leukocytes (Fig. 7Ab vs. a, arrowheads) [100], with a marked increase in its mRNA expression (Fig. 7B).

Previous studies demonstrated the overexpression of Kv1.3 channels in cells under various pathologic conditions, including cancer [105, 106], ischemic heart disease [107], and autoimmune disorders such as rheumatoid arthritis [108] and multiple sclerosis [109]. Concerning the mechanisms involved in such overexpression of the channels, stimulation by transforming growth factor-β (TGF-β) was one of the most likely candidates demonstrated in macrophages [110]. Since uremic toxins, such as indoxyl sulfate, upregulate the expression of TGF-β [111], they might be responsible for the overexpression of the channels in leukocytes in advanced CRF. By generating a driving force for the calcium influx, the membrane hyperpolarization brought about by the opening of Kv1.3-channels stimulates the calcium signaling necessary to trigger the lymphocyte activation [6, 7, 34]. As demonstrated in cancer cells [112], the membrane hyperpolarization induced by the overexpression of the channels was thought to promote the proliferation of leukocytes. In addition to their role in cellular proliferation, Kv1.3 channels expressed in lymphocytes and macrophages trigger cytokine production from those cells [6, 7, 56]. Thus, the overexpression of the channels in leukocytes would have numerous effects on the progression of renal fibrosis.

Therapeutic effects of selective Kv1.3-channel inhibitors on the progression of CKD

To obtain direct evidence that Kv1.3 channels expressed in lymphocytes actually contribute to the development of renal diseases, previous studies therapeutically used selective Kv1.3-channel inhibitors, such as ShK and Psora-4 [88, 89]. Margatoxin is one of the potent inhibitors of Kv1.3 channels and almost totally inhibits the channel currents in lymphocytes [40]. In experimental animal models with lung carcinoma [113], this toxin exerted antitumor activity without showing any neuromuscular side effects [114, 115]. In our study, therefore, margatoxin was used for the treatment of CKD for the first time (Fig. 8) [100]. In advanced CRF rat kidneys with margatoxin treatment, the size of the cortical interstitium was smaller, and the number of infiltrating leukocytes was much smaller (Fig. 8Ab vs. a). Additionally, in those kidneys, immunohistochemistry for collagen III, a marker of fibrosis, demonstrated less staining in the cortical interstitium (Fig. 8Bb vs. a), indicating that margatoxin ameliorated the progression of renal fibrosis. From these results, the overexpression of Kv1.3 channels in kidney lymphocytes was thought to promote their cellular proliferation in advanced CKD (Fig. 9). The proliferating lymphocytes were thought to be responsible for the progression of renal fibrosis, possibly through the increased cytokine production and the fibroblast activation (Fig. 9).

Grbic et al. [116] demonstrated the therapeutic efficacy of blocking the intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) channels.
channels (K_{c,3.1}) for renal fibrosis, since fibroblasts overexpressed the channels under a pathologic condition. In a separate animal study, they also demonstrated the prophylactic efficacy of blocking lymphocyte Kv1.3 channels for kidney allograft rejection [88]. As an extension of these studies, our study further suggested that targeting the Kv1.3 channels overexpressed in leukocytes would also be useful for the treatment of renal fibrosis in advanced CRF [100]. In our series of patch-clamp studies, in addition to NSAIDs, macrolide antibiotics, and CCBs [41–43], HMG-CoA reductase inhibitors (statins) also effectively suppressed lymphocyte Kv1.3-channel currents [117]. Therefore, in this regard, besides the use of the previously developed selective blockers for the channels [44, 118], the use of these drugs could also potentially be useful as anti-fibrotic agents in patients with advanced CKD [40–43].

**Overview and future directions**

T lymphocytes predominantly express Kv1.3 channels in their plasma membranes. Patch-clamp studies showed physiological evidence that some commonly used drugs, such as NSAIDs, macrolide antibiotics, and CCBs, suppress the channel currents in lymphocytes, and thus exert immunosuppressive effects.

According to animal studies using rat models with advanced CRF, the overexpression of lymphocyte Kv1.3 channels contributed to the progression of renal fibrosis by promoting cellular proliferation. Since the channel inhibition actually ameliorated the progression of the disease, this channel could be a potent therapeutic target for advanced CKD and possibly for other “chronic inflammatory diseases”. Several in-vitro studies, including ours, strongly suggested the therapeutic usefulness of potent Kv1.3-channel inhibitors, such as NSAIDs, macrolide antibiotics, and CCBs, in the treatment or the prevention of CKD.

Besides lymphocytes, Kv1.3 channels are also expressed in other types of hematopoietic cells, such as megakaryocytes [81, 82, 119], and in various types of cancer cells [105, 106]. Therefore, our future tasks would include revealing the as of yet unknown significance of the channels in a variety of physiological or pathologic conditions, such as thrombopoiesis and tumor progression.

**Conflict of interest** The author declares that he has no conflict of interest.

**References**

1. Cahalan MD, Chandy KG, DeCoursey TE, Gupta S (1985) A voltage-gated potassium channel in human T lymphocytes. J Physiol 358:197–237

2. DeCoursey TE, Chandy KG, Gupta S, Cahalan MD (1984) Voltage-gated K⁺ channels in human T lymphocytes: a role in mitogenesis? Nature 307:465–468

3. Lewis RS, Cahalan MD (1995) Potassium and calcium channels in lymphocytes. Annu Rev Immunol 13:623–653

4. Ishida Y, Chused TM (1993) Lack of voltage sensitive potassium channels and generation of membrane potential by sodium potassium ATPase in murine T lymphocytes. J Immunol 151:610–620

5. Deutsch C, Chen LQ (1993) Heterologous expression of specific K⁺ channels in T lymphocytes: functional consequences for volume regulation. Proc Natl Acad Sci USA 90:10036–10040

6. Cahalan MD, Wulff H, Chandy KG (2001) Molecular properties and physiological roles of ion channels in the immune system. J Clin Immunol 21:235–252

7. Hu L, Pennington M, Jiang Q, Whartenby KA, Calabresi PA (2007) Characterization of the functional properties of the voltage-gated potassium channel Kv1.3 in human CD4+ T lymphocytes. J Immunol 179:4563–4570

8. Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA et al (2004) K⁺ channels as targets for specific immunomodulation. Trends Pharmacol Sci 25:280–289

9. Sugiyama K, Isogai K, Toyama A, Satoh H, Saito K et al (2011) Correlation between the pharmacological efficacy of cyclosporine and tacrolimus as evaluated by the lymphocyte immunosuppressant sensitivity test (LIST) and the MTT assay procedure in patients before and after renal transplantation. Int J Clin Pharmacol Ther 49:145–152

10. Rangaraju S, Chi V, Pennington MW, Chandy KG (2009) Kv1.3 potassium channels as a therapeutic target in multiple sclerosis. Expert Opin Ther Targets 13:909–924

11. Cho JY (2007) Immunomodulatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) at the clinically available doses. Arch Pharm Res 30:64–74

12. Sugiyama K, Shirai R, Muke H, Ishimoto H, Nagata T et al (2007) Differing effects of clarithromycin and azithromycin on cytokine production by murine dendritic cells. Clin Exp Immunol 147:540–546

13. Morikawa K, Zhang J, Nonaka M, Morikawa S (2002) Modulatory effect of macrolide antibiotics on the Th1- and Th2-type cytokine production. Int J Antimicrob Agents 19:53–59

14. Khan AA, Slifer TR, Araujo FG, Remington JS (1999) Effect of clarithromycin and azithromycin on production of cytokines by human monocytes. Int J Antimicrob Agents 11:121–132

15. Palamaras I, Kyriakis K (2005) Calcium antagonists in dermatology: a review of the evidence and research-based studies. Dermatol Online J 11:8

16. Horvath M, Mezey Z, Jofyay A, Nanay I, Varsanyi M et al (1991) The effect of some drugs on in vitro cellular immune reactions and on circulating immune complexes in patients with myocardial infarction. J Investig Allerg Clin Immunol 1:404–410

17. Zhou Y, Hancock JF, Lichtenberger LM (2010) The nonsteroidal anti-inflammatory drug indomethacin induces heterogeneity in lipid membranes: potential implication for its diverse biological action. PLoS One 5:e8811

18. Zuckerman JM (2004) Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. Infect Dis Clin N Am 18:621–649, xi.

19. Chester DW, Herbeste LG, Mason RP, Joslyn AF, Triggel DJ et al (1987) Diffusion of dihydropyrudine calcium channel antagonists in cardiac sarclemmal lipid multibilayers. Biophys J 52:1021–1030

20. Young HS, Skita V, Mason RP, Herbeste LG (1992) Molecular basis for the inhibition of 1,4-dihydropyridine calcium channel drugs binding to their receptors by a nonspecific site interaction mechanism. Biophys J 61:1244–1255
21. Heap GA, van Heel DA (2009) The genetics of chronic inflammatory diseases. Hum Mol Genet 18:R101–R106
22. Kazama I, Maruyama Y, Baba A (2014) Amphiphath-induced plasma membrane curvature controls microparticle formation from adipocytes: novel therapeutic implications for metabolic disorders. Med Hypotheses 82:196–198
23. Bohle A, Strutz F, Muller GA (1994) On the pathogenesis of chronic renal failure in primary glomerulopathies: a view from the interstitium. Exp Nephrol 2:205–210
24. Strutz F, Zeisberg M (2006) Renal fibroblasts and myofibroblasts in chronic kidney disease. J Am Soc Nephrol 17:2992–2998
25. Michimata M, Kazama I, Mizukami K, Araki T, Nakamura Y et al (2003) Urinary concentration defect and limited expression of sodium cotransporter, NBSC1, in a rat model of chronic renal failure. Nephron Physiol 93:34–41
26. Suzuki K, Hatanou R, Michimata M, Kazama I, Suzuki M et al (2005) Residual urinary concentrating ability and AQP2 expression in a rat model for chronic renal failure. Nephron Physiol 99:16–22
27. Sanada S, Toyama H, Ejima Y, Matsubara M (2009) Potential for erythropoietin synthesis in kidney of uraemic rat alters depending on severity of renal failure. Nephrology (Carlton) 14:735–742
28. Kumano K, Kogure K, Tanaka T, Sakai T (1986) A new method of inducing experimental chronic renal failure by cryosurgery. Kidney Int 30:433–436
29. Jones SE, Kelly DJ, Cox AJ, Zhang Y, Gow RM et al (2003) Mast cell infiltration and chemokine expression in progressive renal disease. Kidney Int 64:906–913
30. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G (2005) Biomarkers of inflammation and progression of chronic kidney disease. Kidney Int 30:433–436
31. Kaysen GA, Kumar V (2003) Inflammation in ESRD: causes and potential consequences. J Ren Nutr 13:158–160
32. Barreto DV, Barreto FC, Liableu S, Temmar M, Lemke HD et al (2010) Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. Kidney Int 77:550–556
33. Cahalan MD, Chandy KG (2009) The functional network of ion channels in T lymphocytes. Immunol Rev 231:59–87
34. Cahalan MD, Chandy KG (2009) Mitogen-induced oscillations of cytosolic Ca²⁺ and transmembrane Ca²⁺ current in human leukemic T cells. Cell Regul 19:99–112
35. Lewis RS, Cahalan MD (1989) Mitogen-induced oscillations of cytosolic Ca²⁺ and transmembrane Ca²⁺ current in human leukemic T cells. Cell Regul 1:99–112
36. Lewis RS, Ross PE, Cahalan MD (1993) Chloride channels activated by osmotic stress in T lymphocytes. J Gen Physiol 101:801–826
37. Kozak JA, Matsushita M, Nairn AC, Cahalan MD (2005) Charge screening by internal pH and polyanion cations as a mechanism for activation, inhibition, and rundown of TRPM7/MIC channels. J Gen Physiol 126:499–514
38. Kazama I, Maruyama Y, Murata Y, Sano M (2012) Voltage-dependent biphasic effects of chloroquine on delayed rectifier K(+) channel currents in murine thymocytes. J Physiol Sci 62:267–274
39. Kazama I, Maruyama Y, Murata Y (2012) Suppressive effects of nonsteroidal anti-inflammatory drugs diclofenac sodium, salicylate and indomethacin on delayed rectifier K⁺ channel currents in murine thymocytes. Immunopharmacol Immunotoxicol 34:874–878
40. Kazama I, Maruyama Y (2013) Differential effects of clarithromycin and azithromycin on delayed rectifier K(+) channel currents in murine thymocytes. Pharm Biol 51:760–765
41. Kazama I, Maruyama Y, Matsubara M (2013) Benidipine persistently inhibits delayed rectifier K(+) channel currents in murine thymocytes. Immunopharmacol Immunotoxicol 35:28–33
42. Hamilton DL, Beall C, Jeromson S, Chevzoff C, Guthbertson DJ et al (2014) Kv1.3 inhibitors have differential effects on glucose uptake and AMPK activity in skeletal muscle cell lines and mouse ex vivo skeletal muscle. J Physiol Sci 64:13–20
43. Han S, Yi H, Yin SJ, Chen ZY, Liu H et al (2008) Structural basis of a potent peptide inhibitor designed for Kv1.3 channel, a therapeutic target of autoimmune disease. J Biol Chem 283:19058–19065
44. Chandy KG, Cahalan M, Pennington M, Norton RS, Wulff H et al (2001) Potassium channels in T lymphocytes: toxins to therapeutic immunosuppressants. Toxicon 39:1269–1276
45. Mouhat S, Teodorescu G, Homerick D, Visan V, Wulff H et al (2006) Pharmacological profiling of Orthochirus scrobiculosis toxin 1 analogs with a trimmed N-terminal domain. Mol Pharmacol 69:354–362
46. Orban C, Biro E, Grozics E, Bajnok A, Toldi G (2013) Modulation of T lymphocyte calcium influx patterns via the inhibition of kv1.3 and ikca1 potassium channels in autoimmune disorders. Front Immunol 4:234
47. Beeton C, Wulff H, Barbara J, Clot-Faybesse O, Pennington M et al (2001) Selective blockade of T lymphocyte KCa1.5 channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. Proc Natl Acad Sci USA 98:13942–13947
48. Gotzsche PC (1989) Methodology and overt and hidden bias in reports of 196 double-blind trials of nonsteroidal antiinflammatory drugs in rheumatoid arthritis. Control Clin Trials 10:31–56
49. Wallace DJ (2010) Advances in drug therapy for systemic lupus erythematosus. BMC Med 8:77
50. Kazama I, Sasagawa N, Nakajima T (2012) Complete remission of human parvovirus b19 associated symptoms by loxoprofen in patients with atopic predispositions. Case Rep Med 2012:703281
51. Inguez MA, Punzon C, Fresno M (1999) Induction of cyclooxygenase-2 on activated T lymphocytes: regulation of T cell activation by cyclooxygenase-2 inhibitors. J Immunol 163:111–119
52. Hackstein H, Morelli AE, Larregina AT, Ganster RW, Papworth GD et al (2001) Aspirin inhibits in vitro maturation and in vivo immunostimulatory function of murine myeloid dendritic cells. J Immunol 166:7053–7062
53. Gao JX, Issekutz AC (1993) The effect of ebselen on polytherapeutic collagenase. J Orthochirus scrobiculosus (2006) Pharmacological profiling of Therapeutic immunosuppressants. Toxicon 39:1269–1276
potentially adverse response to renal ablation. Am J Physiol 241:F85–F93
98. Brenner BM, Meyer TW, Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 307:652–659
99. Kohzuki M, Kanazawa M, Liu PF, Kamimoto M, Yoshida K et al (1995) Kinin and angiotensin II receptor antagonists in rats with chronic renal failure: chronic effects on cardio- and renoprotection of angiotensin converting enzyme inhibitors. J Hypertens 13:1785–1790
100. Kazama I, Maruyama Y, Endo Y, Toyama H, Ejima Y et al (2012) Overexpression of delayed rectifier K(+) channels promotes in situ proliferation of leukocytes in rat kidneys with advanced chronic renal failure. Int J Nephrol 2012:581581
101. El Nahas AM, Anderson S, Harris KPG (eds) (2000) Mechanisms and management of progressive renal failure. Oxford University Press, London, pp 104–145
102. Litjens NH, van Druningen CJ, Betjes MG (2006) Progressive loss of renal function is associated with activation and depletion of naive T lymphocytes. Clin Immunol 118:83–91
103. Lan HY, Nikolic-Paterson DJ, Mu W, Atkins RC (2004) A voltage-gated K(+) current in renal inner medullary collecting duct cells. Am J Physiol Cell Physiol 286:C965–C974
104. Abdul M, Hoosein N (2002) Expression and activity of potassium ion channels in human prostate cancer. Cancer Lett 186:99–105
105. Jang SH, Kang KS, Ryu PD, Lee SY (2009) Kv1.3 voltage-gated K(+) channel subunit as a potential diagnostic marker and therapeutic target for breast cancer. BMB Rep 42:535–539
106. Huang S, Zhang CT, Tang JR, Tang J, Cai L, Zhang Z, Zhou MG (2010) Upregulated voltage-gated potassium channel Kv1.3 on CD4+CD28null T lymphocytes from patients with acute coronary syndrome. J Geriatr Cardiol 7:40–46
107. Toldi G, Bajnok A, Dobi D, Kaposi A, Kovacs L et al (2013) The effects of Kv1.3 and IKCa1 potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis. Immunobiology 218:311–316
108. Rus H, Pardo CA, Hu L, Darrah E, Cudrici C et al (2005) The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. Proc Natl Acad Sci USA 102:11094–11099
109. Schilling T, Eder C (2003) Effects of kinase inhibitors on TGF-beta induced upregulation of Kv1.3 K(+) channels in brain macrophages. Pflugers Arch 447:312–315
110. Miyazaki T, Ise M, Seo H, Niwa T (1997) Indoxyl sulfate increases the gene expressions of TGF-beta 1, TIMP-1 and pro-alpha 1(I) collagen in uremic rat kidneys. Kidney Int Suppl 62:S15–S22
111. El Nahas AM, Anderson S, Harris KPG (eds) (2000) Mechanisms and management of progressive renal failure. Oxford University Press, London, pp 104–145
112. Lewis RJ, Garcia ML (2003) Therapeutic potential of venom peptides. Nat Rev Drug Discov 2:790–802
113. Judge SI, Lee JM, Bever CT Jr, Hoffman PM (2006) Voltage-gated potassium channels in multiple sclerosis: overview and new implications for treatment of central nervous system inflammation and degeneration. J Rehabil Res Dev 43:111–122
114. Jang SH, Choi SY, Ryu PD, Lee SY (2011) Anti-proliferative effect of Kv1.3 blockers in A549 human lung adenocarcinoma in vitro and in vivo. Eur J Pharmacol 651:26–32
115. Schmitz A, Sankaranarayanan A, Azam P, Schmidt-Lassen K, Homeric D et al (2005) Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases. Mol Pharmacol 68:1254–1270
116. McCloskey C, Jones S, Amisten S, Snowden RT, Kaczmarek LK et al (2010) Kv1.3 is the exclusive voltage-gated K(+) channel of platelets and megakaryocytes: roles in membrane potential, Ca(2+) signalling and platelet count. J Physiol 588:1399–1406