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Toxins Targeting the Kv1.3 Channel: Potential Immunomodulators for Autoimmune Diseases

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Abstract: Autoimmune diseases are usually accompanied by tissue injury caused by autoantigen-specific T-cells. Kv1.3 channels participate in modulating calcium signaling to induce T-cell proliferation, immune activation and cytokine production. Effector memory T (TEM)-cells, which play major roles in many autoimmune diseases, are controlled by blocking Kv1.3 channels on the membrane. Toxins derived from animal venoms have been found to selectively target a variety of ion channels, including Kv1.3. By blocking the Kv1.3 channel, these toxins are able to suppress the activation and proliferation of TEM cells and may improve TEM cell-mediated autoimmune diseases, such as multiple sclerosis and type I diabetes mellitus.

Keywords: toxins; Kv1.3 channel; effector memory T-cell; autoimmune diseases
1. Introduction

Voltage-gated ion channels function as guards with regard to the ion permeability of cell membranes to regulate the intracellular concentrations of various ions, such as potassium (K\(^+\)), sodium (Na\(^+\)) and calcium (Ca\(^{2+}\)). Because of selective evolution, venomous animals and plants have the ability to produce toxins targeted at these ion channels that cause paralysis, respiratory failure and death in their enemies. Peptide toxins from snakes, scorpions, spiders, sea anemones and cone snails have been widely used for structural and functional research addressing ion channels [1]. Therefore, there is a great potential for applying peptide toxins in designing drugs for ion channel diseases.

In the last few years, progress has been made in identifying the types of ion channels in lymphocytes and their roles in regulating Ca\(^{2+}\) signaling, activation, differentiation, secretion and migration.

The focus of potassium channels that regulate membrane potential in lymphocytes is the Shaker-related voltage-gated K\(^+\) channel KV1.3 (also known as KCNA3) and the Ca\(^{2+}\)-activated K\(^+\) channel KCa3.1 (also known as IKCa1 or KCNN4). The expression levels of KV1.3 and KCa3.1 and their contributions to Ca\(^{2+}\) influx are different based on the types and activated state of T-cells. In autoimmune disorders, effector memory T (TEM)-cells encounter self-antigens and are activated, and the KV1.3 channels on the cell membrane increase significantly. Toxins targeting KV1.3 can selectively impact TEM cell function, which suggests the regulatory role of KV1.3-blocking toxins in autoimmune diseases [2].

2. T-Cells and Autoimmune Diseases

An immune system distinguishes infectious pathogens from its own body components by a complex network. In general, most animals do not induce immunoreactions to autoantigens, forming self-tolerance. However, under certain conditions, an immune system will provoke positive immune responses to autoantigens. When the balance of immunoreaction and immunoregulation breaks down, autoimmune diseases may develop. Memory T-cells play a major role in the progression of these diseases.

Based on expression levels of chemokine CCR7 and phosphatase CD45RA, human memory cells can be sorted into central memory T (T\(_{CM}\)) and TEM cells. Both naive CD4\(^+\) and CD8\(^+\) cells express CCR7 and CD45RA and enter into lymph nodes with CCR7. In lymph nodes, naive T-cells are activated by antigens presented by antigen-presenting cells (APCs), release cytokines and differentiate into effector cells. When antigens are eliminated, most effector cells will undergo programmed cell death, but a few cells can survive and differentiate into long-lived T\(_{CM}\) cells. These cells also rely on CCR7 for entry into lymph nodes, but do not express CD45RA. When T\(_{CM}\) cells recognize the same antigens, they turn into effector cells and clear antigens more efficiently. In autoimmune diseases or chronic infections, antigens continually stimulate the immune system, and T\(_{CM}\) cells differentiate into TEM cells. These cells live for a short time and develop into CCR7\(^-\)CD45RA\(^-\) (exception: CD8\(^+\)CCR7\(^-\)CD45RA\(^+\)TEMRA). They do not need to enter into lymph nodes and remain in the periphery to be ready for stimulation. When an antigen is encountered, TEM cells differentiate into effector T-cells and migrate to an inflammatory site. Then, they release pro-inflammatory factors for a quick immune response. CD8\(^+\) TEM cells also serve as cytotoxic T lymphocytes and release cytotoxicity factors, such as perforin and granzyme [3–5].

When a thymus matures, most autoreactive T-cells are eliminated, but a few cells escape to peripheral immune organs. Under normal conditions, these cells are suppressed by regulatory T-cells. In autoimmune
diseases, this suppression mechanism disappears, and autoreactive T-cells attack target organs, causing tissue injury.

For example, multiple sclerosis (MS) is a typical autoimmune disease in which autoreactive T-cells migrate from peripheral blood into the CNS and attack axons, myelin sheaths and oligodendrocytes. This damage leads to demyelination and impacts neural signal transmission, which causes CNS dysfunction. The key event is when inflammatory T-cells permeate the blood brain barrier (BBB), invade CNS and are re-stimulated by autoantigens myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) or myelin oligodendrocyte glycoprotein [6,7].

3. **KV1.3 and Immune System Regulation**

3.1. **KV1.3 and KCa3.1 in the Immune System**

Potassium channels are tetrameric membrane proteins that mediate K⁺ efflux to hyperpolarize the cellular membrane. Made up of 78 family members, the K⁺ channel family is the third largest family of signaling molecules in the human genome, following G-protein coupled receptors and protein kinases [8]. They are expressed on cells from both innate and adaptive immune systems.

The innate immune system includes natural killer (NK) cells, mast cells, eosinophils, basophils, and the phagocytic cells monocytes/macrophages, neutrophils and dendritic cells (DCs). These cells recognize “invaders” and release toxic molecules to non-specifically kill them. Moreover, macrophages and DCs, which present antigens to T-cells and play roles in adaptive immunity, are called antigen-presenting cells. Based on the published literature, most innate immune cells express K⁺ channels, but their functions in different cell types require further research.

In contrast, K⁺ channel expression has been systematically elucidated in the adaptive immune system. Patch clamping makes recording electrical signals from a single lymphocyte possible. Kv1.3 and KCa3.1 expression levels are detected in the membranes of T-cells. The expression levels of these two potassium channels track the activation and differentiation of T-cells [9–11]. However, there are some differences in their mediation of the efflux of K⁺.

Kv1.3 consists of four α-subunits arranged around a central pore as a homotetramer. Each subunit is composed of six transmembrane segments S1–S6 and a pore loop between S5 and S6. When membrane depolarization occurs, the arginine residues present in the S4 segment act as voltage sensors and cause a structure change and channel opening. In addition to T-cells, the Kv1.3 channel is also expressed in other immune cells, such as B-cells [12] and macrophages [13].

KCa3.1, expressed in lymphoid organs, is a calcium-dependent K⁺ channel, the C-terminus of which binds to calmodulin. When Ca²⁺ interacts with calmodulin, the channel opens and strongly hyperpolarizes the membrane, which provides a force for Ca²⁺ entry [2,14].

Each of these K⁺ channels regulate the membrane potential of T-cells and assist calcium release-activated calcium (CRAC) channels in promoting calcium influx, which is essential for T-cell activation. When an immune response occurs, an APC presents an antigen to a TCR. Then, IP₃ is produced and binds to the IP₃R, which releases Ca²⁺ from the endoplasmic reticulum (ER). Meanwhile, stromal interaction molecule 1 (STIM1) or STIM2 activates the opening of CRAC channels for Ca²⁺ entry. The Ca²⁺ influx results in gene expression through the calcineurin-NFAT pathway. The opening
of Kv1.3 and KCa3.1 induces the efflux of K⁺ to counterbalance the increase of Ca²⁺ in the cytoplasm [8,15] (Figure 1).

**Figure 1.** The influx of Ca²⁺ and efflux of K⁺ in the stimulation of memory T-cell. After, APC presents antigen to a T-cell, and IP₃ is produced, which liberates Ca²⁺ from the ER. Meanwhile, CRAC channels are open and lead a Ca²⁺ influx. The increase of Ca²⁺ promotes the efflux of K⁺.

**Figure 2.** Expressions of Kv1.3 and KCa3.1 channels in human T-cells. The expressions of both channels vary based on states of activation and differentiation. (Eff-T cell is effector T cell for short.)
The contribution of Kv1.3 or KCa3.1 to the intracellular Ca\(^{2+}\) concentration is determined by their expression levels and varies in different subsets of T-cells. When human T-cells are inactive, they express 200–300 Kv1.3 and 5–20 KCa3.1 per cell [16,17]. Following stimulation by antigens or mitogens, channel expression differences between effector T-cells arise. In naive or T\(_{CM}\) cells, the transcription and translation of KCa3.1 are significantly upregulated, and the number on the membrane can be increased to the levels of Kv1.3 (KCa3.1 200–500, Kv1.3 400–500 per cell) [16]. However, while Kv1.3 expression increases (1,500 per cell), KCa3.1 levels stay nearly constant in T\(_{EM}\) cells [17] (Figure 2). This causes T\(_{EM}\) cells to produce various cytokines, including IL-2, IFN-\(\gamma\), IL-4 and IL-17. These variances in expression affect membrane potential and calcium signaling, which regulate the expression of the downstream gene for T-cell activation and immune responses. As a result, targeting the Kv1.3 channel inhibits T\(_{EM}\) cells activated by autoantigens in inflammation sites and is a promising strategy in remitting autoimmune diseases.

### 3.2. Kv1.3 Channel Inhibitors

K\(^{+}\) channels mediate the efflux of K\(^{+}\) to hyperpolarize the plasma membrane when the membrane potential is depolarized. The \(\alpha\)-subunits of K\(^{+}\) channels gather to form a heterotetramer relatively freely and are responsible for the different biophysical and pharmacological properties of cells. The \(\beta\)-subunits can interact with and modify the \(\alpha\)-subunit complexes [18]. K\(^{+}\) channel proteins can also be post-translationally modified by mechanisms, such as phosphorylation, ubiquitinylation and palmitoylation.

The diversity of the structural characteristics of the K\(^{+}\) channel family causes great challenges in drug discovery. However, this diversity is also promising for the highly selective targeting of specific types of homotetramers or heterotetramers.

There are three strategies for K\(^{+}\) channel regulator design: metal ions, organic small molecules and venom-derived peptides. The mechanism generally targeted is blocking the central pore of K\(^{+}\) channels to prevent K\(^{+}\) transport or modifying the structure of the voltage-sensor domain. For metal ions, such as Ba\(^{2+}\), Co\(^{2+}\) and Ni\(^{2+}\), the effect on K\(^{+}\) channel blocking is small and not selective. Thus, there is less focus on the metal ion strategy. The other two types of drugs can be used to treat immune system and nervous system diseases. In addition, toxins have advantages over small molecules due to their affinity and selectivity for specific channels. Toxins and their analogs can inhibit one type of potassium channels at the picomole level and display 1,000-fold selectivity for it over other potassium channels. The extremely high affinity and selectivity of toxins are difficult to achieve with chemically-designed molecules.

Venomous animals, such as snakes, scorpions, spiders, sea anemones and cone snails, can produce a variety of toxins that act on ion channels. The first toxin reported in the literature was the bee neurotoxin apamin, which blocks the KCa2 (SK) channel [19,20]. Subsequently, the scorpion toxins noxiustoxin (NTX) [21] and charybdotoxin (ChTX) [22] were discovered. NTX blocks Kv1.2 (IC\(_{50} \approx 2\) nM) and Kv1.3 (IC\(_{50} \approx 1\) nM), two members of the Kv1 family. While the selectivity of ChTX is poor, it inhibits KCa1.1 in nM concentrations [22], as well as Kv1.2 (IC\(_{50} \approx 14\) nM), Kv1.3 (IC\(_{50} \approx 2.6\) nM) and Kv1.6 (IC\(_{50} \approx 22\) nM) [23]. These toxins are applied in neuroscience to increase the physiological properties of K\(^{+}\) channels. However, their potential can be further exploited. These peptides may help us better study the structure of channel proteins, the composition of the subunits and the mechanism underlying K\(^{+}\) gating.
In the past 30 years, hundreds of toxin peptides targeting K+ channels have been discovered. The toxins contain between 18 and 60 amino acid residues and are assembled with 2–4 disulfide bridges, which makes them resistant to denaturation [24]. These peptides and their synthetic derivatives target KV and KCa channels efficiently and selectively. However, Kir- and K2P channel-blocking toxins are rarely reported [25,26].

The peptides modify K+ channels through two mechanisms. Toxins from scorpions, sea anemones, snakes and cone snails bind the outer vestibule of the channels and close the central pore by inserting a lysine side chain into it, similar to plugging a cork into a wine bottle; subsequently, peptides interact with the outside channel amino acids to stabilize their combination [8,27–29] (Figure 3). Spider toxins act on the voltage sensor domain of K+ channels, making the channel more difficult to open and increasing the possibility of a closed state [30,31]. As modified gating toxins, spider toxins bind to the voltage sensor domain and insert one side of a cluster of hydrophobic residues into the membrane to strengthen their bindings, but the insertion has no specificity, making drug design difficult (Figure 3).

**Figure 3.** The mechanism of animal toxins blocking the potassium channel. Potassium channels function as a heterotetramer. Most toxins from animal venoms block the central pore to prevent K+ transport, while spider toxins regulate the voltage sensors.

Our goal is to search for KV1.3 blockers with potential as immunomodulators in autoimmune diseases. The first compound used for KV1.3 function research was 4-aminopyridine (4-AP). Although it blocks KV1.3 well and has been used in the clinic to treat MS, its poor selectivity leads to side effects, such as headaches and seizures [32]. Low selectivity is a common limitation of small molecules for the intended target. Submicromolar inhibitors of KV1.3 are also being explored and synthesized. The therapeutic blockers with the most potential focus on toxin peptides from scorpions and sea anemones because of their high affinities and selectivities.

Toxins derived from scorpion venom, sea anemone, snakes and other animals were found to block KV1.3 with different affinities and selectivities. Among these toxins, there are numerous scorpion toxins.
targeting Kv1.3, including noxiustoxin (NTX), charybdotoxin (ChTX), margatoxin (MgTX), Orthochirus scrobiculosus toxin 1 (OSK1), kaliotoxin, agitoxin-2, hongotoxin and anuroctoxin [8]. As research progresses, more Kv1.3-blocking toxins from various animals are being discovered that block Kv1.3 with various IC50 values (Table 1).

Table 1. Toxins blocking the Kv1.3 channel. The blocking potencies (Kd or IC50 values) of wild-type toxins and selected analogs on Kv1.3 channels are shown. For comparison, Kv1.1 and Kv1.2 channels are included for selectivity.

| Toxins WT/Analog | Derived from | Kv1.3 Kd/IC50 (nM) | Kv1.1 Kd/IC50 (nM) | Kv1.2 Kd/IC50 (nM) | Reference |
|-----------------|-------------|--------------------|--------------------|--------------------|-----------|
| Noxiustoxin     | Scorpion    | 1                  | >25                | 2                  | [33]      |
| Charybdotoxin   | Scorpion    | 2.6                | >1,000             | 14                 | [33]      |
| Margatoxin      | Scorpion    | 0.11               | 0.52               | 10                 | [34]      |
| Kaliotoxin      | Scorpion    | 0.41               | 1.1                | 25                 | [35]      |
| Agitoxin-2      | Scorpion    | 0.004              | 0.044              | ND                 | [36]      |
| Hongotoxin      | Scorpion    | 86                 | 31                 | 170                | [37]      |
| Anuroctoxin     | Scorpion    | 0.73               | >100               | 6.1                | [38]      |
| OSK1            | Scorpion    | 0.003              | 0.4                | 2.96               | [39]      |
| Vm24            | Scorpion    | 0.029              | 35                 | 7.5                | [40]      |
| ADWX-1          | Scorpion    | 0.002              | 0.65               | >100               | [41]      |
| ImKTX88         | Scorpion    | 0.091              | 389                | 8,470              | [42]      |
| MTX-HsTX1       | Scorpion    | 4                  | 1                  | >100               | [43]      |
| ShK-Dap22       | Sea anemone | 0.023              | 1.8                | 39                 | [44]      |
| ShK-186         | Sea anemone | 0.069              | 7                  | 48                 | [44]      |
| ShK-192         | Sea anemone | 0.14               | 22                 | >100               | [44]      |
| Gambierol       | Dinoflagellate | 853.5             | 64.2               | 34.5               | [45]      |
| ViTX            | Cone snail  | 2,090              | 1,590              | ND                 | [46]      |
| AcK1            | Worm        | 266                | >1,500             | ND                 | [47]      |
| BmK1            | Worm        | 6,900              | ND                 | ND                 | [47]      |
| BF9             | Snake       | 120                | ND                 | ND                 | [48]      |
| Aurelin         | Jellyfish   | 4,120              | ND                 | ND                 | [47]      |

Abbreviation: OSK1, Orthochirus scrobiculosus toxin 1; Vm24, venom toxin 24 from Vaejovis mexicanus smithi; ADWX-1, autoimmune drug 1 from Wexin group; ImKTX88, Isometrus maculates potassium channel toxin 88; MTX, maurotoxin; HsTX1, Heterometrus spiniferr (Scorpionidae) toxin 1; ShK, Stichodactyla helianthus toxin; Dap, Diaminopropionic acid; ViTX, Conus virgo toxin; AcK1, Ancylostoma caninum potassium channel toxin 1; BmK1, Brugia malayi potassium channel toxin 1; BF9, Bungarus fasciatus toxin 9; ND, not detected.

Although the majority of peptides can block Kv1.3 in the nanomolar to picomolar range, their selectivities for the channel are not satisfactory. In order to achieve better results, chemical modifications and point mutation techniques are being applied to the original toxin molecules. The first analysis of these molecules will investigate which residues of the toxins should be modified for higher selectivity binding.

For example, ShK, a peptide from the Caribbean sea anemone, was found to have a high affinity for Kv1.3 (Kd ≈ 11 pM) [49]. Additionally, it blocks the Kv1.1, Kv1.4 and Kv1.6 channels at picomolar concentrations. Subsequently, using a thermodynamic mutant cycle analysis to study residues in toxin...
binding to the mKv1.3 channel, ShK-Dap22 was designed. A non-natural amino acid known as diaminopropionic acid was substituted for lysine22, which is known to be critical for the binding of many toxins. Although this mutant has a lower affinity for mKv1.3 ($K_d \approx 23$ pM) compared to ShK, its selectivity over the other Kv1.x channels and IKCa1 was remarkably improved [50]. On this basis, a further study revealed the different mechanisms controlling the binding of these two toxins to mKv1.3. The wild-type toxin inserts Lys22 into the central pore and interacts with residue Tyr400; this strong interaction was replaced by Dap22 and His402 in the ShK-Dap22 binding. Because of the weak affinity of the interaction between Dap22 and the residues of other channels (i.e., Kv1.1, Kv1.4, Kv1.6) compared to Lys22, ShK-Dap22 has a high selectivity for Kv1.3 [51]. This reveals that minute differences in amino acids may lead to pronounced changes in dynamics. Besides affinity and selectivity, stability also needs to be taken into account. ShK-186 retains the selectivity and potency profile of ShK analogs and minimizes digestion by carboxypeptidases by replacing the C-terminal carboxyl with an amide [44].

In addition to small-scale changes in residues, toxins can be combined with other regions for special purposes; this requires analyzing the binding domain affinity that can be used for multi-target, high-selectivity drug design. Maurotoxin (MTX) is an effective SK channels blocker, and HsTX1 inhibits Kv1.1 and Kv1.3. MTX-HsTx1 contains the N-terminal helical region of MTX and the C-terminal $\beta$-sheet region of HsTx1 for binding SK channels, mKv1.1 and mKv1.3 [43].

4. Kv1.3 Blocking Toxins for the Therapy of Autoimmune Diseases

In general, autoimmune diseases involve the stimulation of T-cells at a peripheral region and a target site. The occurrence and progression of these processes are difficult to pinpoint clinically because of tracking, observation and sample collection difficulties. Animal models may help us to better understand these diseases and aid in future drug designs.

However, to test Kv1.3 blocking, the practicability of mice models is limited. T-cells of mice express fewer and different potassium channels [52,53]. When stimulated, the numbers of Kv1.3 and KCa3.1 both increase in T$\text{EM}$ cells (Kv1.3 from 300–800 and KCa3.1 from 130–400) [5], which indicates that the application of Kv1.3 blockers in a mouse model is not convincing for effective T$\text{EM}$ targeting.

Rat autoimmune disease models, by contrast, are more persuasive. Although the expression of potassium channels on T-cell membranes is also different from that of humans in a quiescent state, after stimulation, the rat T$\text{EM}$ phenotype upregulates Kv1.3, and the T$\text{CM}$ phenotype maintains Kv1.3 at low levels. This difference is similar to that in humans, which suggests that rat models are better for evaluating the role of T$\text{EM}$ cells in T-cell-mediated autoimmune diseases and the therapeutic effects of Kv1.3 blockers [49,54].

4.1. Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by myelin damage in the central nervous system (CNS). The clinical symptoms of this disease are decreased vision, eye pains, dysphagia, limb weakness and difficulties in walking. There are millions of patients around the world, the majority of whom are women. The incidence of MS continues to increase. A small number of patients can be cured after the first attack, but most patients have relapsing and remitting disease [7].
Clinical samples from MS patients (brain, spinal cord and cerebrospinal fluid) are not easy to get, which cannot satisfy the research for potential drugs. Therefore, animal models have been generated to study MS. These models fall into three categories: gene mutation or chemically (i.e., cuprizone)-induced white matter lesions for myelination and remyelination research [55], virus-induced models for etiology studies and autoimmune cell-mediated immune responses for neuroinflammation studies. The classic model is experimental autoimmune encephalomyelitis (EAE), in which the imbalance of immune activation and immunoregulation leads to worsening CNS injury; this model has the most similar pathogenesis to MS and is generally used for drug screening [56–58].

In the 1980s, it was reported that after being stung by a scorpion, an MS patient had reduced disease [59]. This observation suggests that peptides from scorpion venom may improve MS. As a typical autoimmune disease, MS and its animal models are regularly studied in research into Kv1.3-blocking toxins.

In the Sprague-Dawley (SD) rat acute EAE model, ADWX-1, an analog of scorpion toxin BmKTX [41], has been shown to exhibit preferential, dose-dependent inhibition of CD4+CCR7− TEM cells, which are perpetrators of EAE. ADWX-1 reduces the number of activated Th1 and Th17 cells and also decreases the secretion of inflammatory factors IL-2, IFN-γ and IL-17. In contrast, it has no effect on regulatory Foxp3+ cells, which are the antagonists of autoreactive cells [6].

ShK-186 [60], which was tested in the Dark Agouti (DA) rat EAE model (an animal model that is more similar to chronic relapsing-remitting multiple sclerosis), can also decrease inflammatory T-cell infiltration into the CNS and the demyelination process [60]. This toxin peptide has been used in clinical trials.

It is important to emphasize the selectivity for Kv1.3 in EAE models. Based on the electrophysiological evidence, the Kv1.1 and Kv1.2 of myelinated axons are located under the myelin sheath [61,62]. Toxin peptides, the nanomolar molecules associated with Kv1.x, can easily enter into the CNS after the BBB has been broken down by inflammatory cells. These peptides may then influence the normal physiological functions of neurons. A goal in this field is to obtain good clinical results without neurological impairment.

4.2. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease with widespread concerns. The joint changes are mediated by the dysregulated and invasive growth of the synovial cells developing into what is referred to as a pannus that overlays and destroys cartilage and bone. CCR7-Kv1.3high TEM cells have been identified from synovial fluid of RA patients, suggesting that Kv1.3 blockers may function as immunomodulators in RA [63].

A chronic arthritis model is DA rats injected with pristane oil, which induces RA [64]. Disease severity in this model worsens with time. The immune regulation of drugs can be evaluated by measuring the degree of ankle welling and detecting inflammatory factors (i.e., TNF-α) in synovial fluid. Subcutaneous injections of ShK-186 at the onset of disease signs can ameliorate the severity of RA, including reducing inflammatory infiltration and tissue damage [63]. ShK-186 has been found to preferentially suppress TEM cells from synovial fluid of RA patients [63]. However, there is no difference when the Kv1.3 blocker margatoxin (MgTX) is applied to peripheral lymphocytes from patients with rheumatoid arthritis or healthy individuals. This is because most T-cells in circulation remain in a less-activated state [65].
4.3. Type I Diabetes Mellitus

Type I diabetes mellitus (TIDM) is caused by a loss of beta cells due to autoimmune-mediated destruction. The target antigens recognized by autoimmune cells include insulin, glutamate decarboxylase (GAD) and membrane proteins of islet beta cells. Severe inflammatory infiltration in the islets of patients with HLA-II molecules expressed on beta cell membranes is characteristic of TIDM.

Biobreeding/Worcester (BB/Wor) rats develop experimental autoimmune diabetes mellitus after 70 days. The incidence can reach 100% in 110 days. 5-(4-phenoxybutoxy)psoralen (PAP-1) is a selective small molecule inhibitor of Kv1.3 [66]. PAP-1 administration decreases the clinical manifestation in diseased rats. The rats have less T-cell and macrophage infiltration and less beta cell destruction [63]. Studies have reported that potassium channels are involved in glucose-stimulated insulin secretion in pancreatic beta cells, and Kv1.3 specifically mediates insulin sensitivity in peripheral target tissues. No increase in basal or insulin-stimulated glucose uptake or adiponectin secretion has been reported in PAP-1-treated rat adipocytes, suggesting the effect of PAP-1 on diseased rats works through immunoregulation [67].

ShK-186 delays the onset of diabetes, but has no influence on disease incidence. This may due to the different pharmacokinetic properties of ShK-186, which has a net basic charge that makes concentrating in the pancreas difficult [44,63].

Lymphocytes from peripheral blood of TIDM patients are more sensitive to Kv1.3 inhibition than healthy individuals, probably because of the enhanced Kv1.3 expression in TIDM patients. An increase of TCM cells has also been detected, suggesting that TIDM lymphocytes remain in a pre-activated state in these patients. However, MgTX also fails in modulating TIDM lymphocytes compared with healthy individuals. This indicates that autoantigen-specific T-cells require further investigation [68].

4.4. Rapidly Progressive Glomerulonephritis

Glomerulonephritis (GN) is usually initiated by infections or self-antigens. Immunostimulatory pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) activate both innate and adaptive immune systems. APCs promote the stimulation and differentiation of CD4 helper cells. CD4 Th1 cells mediate tissue damage through macrophages and basophils, while Th17 cells damage glomeruli directly [69].

In rats with anti-glomerular basement membrane glomerulonephritis (anti-GBM GN), Kv1.3 expression was detected in glomeruli and some tubules. A flow cytometry analysis of kidney cells and immunofluorescence staining of lymphocytes revealed that these cells were CD4$^+$ and that a few were CD8$^+$ Kv1.3$^{\text{high}}$ TEM cells. Moreover, rats injected with the small-molecule Kv1.3 blocker Psora-4 showed less proteinuria and fewer crescentic glomeruli [70], suggesting the potential role of Kv1.3 toxin peptides in the treatment of autoimmune glomerulonephritis.

4.5. Psoriasis

Psoriasis, one of the common inflammatory disorders of the skin, affects approximately 2.0%–2.5% of the world’s population. The most common symptom of psoriasis is patches of thick red skin often covered with silvery scales [71]. Psoriasis plaques and synovial fluid from psoriasis/psoriatic arthritis
(PsA) patients exhibit significantly increased Kv1.3+ memory T-cells compared to non-lesion psoriatic skin, normal skin or peripheral blood lymphocytes. Moreover, treatment with the Kv1.3 blocker PAP-1 inhibits T-cell proliferation in the skin and joints of psoriasis patients and the secretion of cytokines IL-2 and IFN-γ [72].

Transplanted psoriatic plaque skin samples from psoriasis patients onto severe combined immunodeficient (SCID) mice results in the development of psoriasis lesions similarly to patients. When grafted mice are treated with ShK, a significant decrease in Kv1.3+ cells is observed along with a marked therapeutic effect, including a normal appearance of the skin grafts [73]. In addition, PAP-1 can also alleviate psoriasis by reducing the hyperkeratosis, acanthosis and immune cell dermal infiltrates in the plaques [72].

5. Conclusions

Kv1.3 channels mainly regulate calcium signaling during the activation of CCR7− TEM cells and maintain membrane potential by counteracting the influx of Ca2+ with the efflux of K+. Because TEM cells play the major role in tissue injury, mediating chronic autoimmune response in autoimmune diseases by blocking Kv1.3 is an effective way to treat these disorders. Based on this strategy, toxins targeting Kv1.3 have been tested in various animal models of autoimmune diseases and samples from patients.

Although there are numerous toxins targeting Kv1.3, because of poor affinity or selectivity, some of them only have applicability as tools for Kv1.3 characterization research in autoimmune diseases. While numerous electrophysiology studies of new synthetic Kv1.3-blocking toxin peptides are being reported, further in vivo experiments are required. ShK-186, an analog derived from ShK, has been used in clinical trials, suggesting the possibilities for autoimmune disease treatment. Toxin peptide researchers should further study toxins with higher affinity and selectivity through investigations using animal models.

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Author Contributions

X.H. conceived the review idea and the frame. Y.Z. and S.H. completed Part 2&3. J.H. and X.Y. designed Part 4. W.L. and B.P. contributed to table and figure generation and revising the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Banerjee, A.; Lee, A.; Campbell, E.; Mackinnon, R. Structure of a pore-blocking toxin in complex with a eukaryotic voltage-dependent K+ channel. eLife 2013, 2, e00594, doi:10.7554/eLife.00594.
2. Cahalan, M.D.; Wulff, H.; Chandy, K.G. Molecular properties and physiological roles of ion channels in the immune system. *J. Clin. Immunol.* 2001, 21, 235–252.

3. Sallusto, F.; Lenig, D.; Forster, R.; Lipp, M.; Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999, 401, 708–712.

4. Sallusto, F.; Geginat, J.; Lanzavecchia, A. Central memory and effector memory T cell subsets: Function, generation, and maintenance. *Annu. Rev. Immunol.* 2004, 22, 745–763.

5. Beeton, C.; Chandy, K.G. Potassium channels, memory T cells, and multiple sclerosis. *Neuroscientist* 2005, 11, 550–562.

6. Li, Z.; Liu, W.H.; Han, S.; Peng, B.W.; Yin, J.; Wu, Y.L.; He, X.H.; Li, W.X. Selective inhibition of CCR7− effector memory T cell activation by a novel peptide targeting Kv1.3 channel in a rat experimental autoimmune encephalomyelitis model. *J. Biol. Chem.* 2012, 287, 29479–29494.

7. Noseworthy, J.H.; Lucchinetti, C.; Rodriguez, M.; Weinshenker, B.G. Multiple sclerosis. *N. Engl. J. Med.* 2000, 343, 938–952.

8. Wulff, H.; Zhorov, B.S. K+ channel modulators for the treatment of neurological disorders and autoimmune diseases. *Chem. Rev.* 2008, 108, 1744–1773.

9. Cahalan, M.D.; Chandy, K.G. The functional network of ion channels in T lymphocytes. *Immunol. Rev.* 2009, 231, 59–87.

10. DeCoursey, T.E.; Chandy, K.G.; Gupta, S.; Cahalan, M.D. Voltage-gated K+ channels in human T lymphocytes: A role in mitogenesis? *Nature* 1984, 307, 465–468.

11. Matteson, D.R.; Deutsch, C. K channels in T lymphocytes: A patch clamp study using monoclonal antibody adhesion. *Nature* 1984, 307, 468–471.

12. Wulff, H.; Knaus, H.G.; Pennington, M.; Chandy, K.G. K+ channel expression during B cell differentiation: Implications for immunomodulation and autoimmunity. *J. Immunol.* 2004, 173, 776–786.

13. Vicente, R.; Escalada, A.; Villalonga, N.; Texido, L.; Roura-Ferrer, M.; Martin-Satue, M.; Lopez-Iglesias, C.; Soler, C.; Solsona, C.; Tamkun, M.M.; et al. Association of Kv1.5 and Kv1.3 contributes to the major voltage-dependent K+ channel in macrophages. *J. Biol. Chem.* 2006, 281, 37675–37685.

14. Verheugen, J.A.; Vijverberg, H.P. Intracellular Ca2+ oscillations and membrane potential fluctuations in intact human T lymphocytes: Role of K+ channels in Ca2+ signaling. *Cell Calcium* 1995, 17, 287–300.

15. Feske, S.; Skolnik, E.Y.; Prakriya, M. Ion channels and transporters in lymphocyte function and immunity. *Nat. Rev. Immunol.* 2012, 12, 532–547.

16. Ghanshani, S.; Wulff, H.; Miller, M.J.; Rohm, H.; Neben, A.; Gutman, G.A.; Cahalan, M.D.; Chandy, K.G. Up-regulation of the IKCa1 potassium channel during T-cell activation. Molecular mechanism and functional consequences. *J. Biol. Chem.* 2000, 275, 37137–37149.

17. Wulff, H.; Calabresi, P.A.; Allie, R.; Yun, S.; Pennington, M.; Beeton, C.; Chandy, K.G. The voltage-gated Kv1.3 K+ channel in effector memory T cells as new target for MS. *J. Clin. Investig.* 2003, 111, 1703–1713.

18. Gutman, G.A.; Chandy, K.G.; Grissmer, S.; Lazdunski, M.; McKinnon, D.; Pardo, L.A.; Robertson, G.A.; Rudy, B.; Sanguinetti, M.C.; Stuhmer, W.; et al. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev.* 2005, 57, 473–508.
19. Banks, B.E.; Brown, C.; Burgess, G.M.; Burnstock, G.; Claret, M.; Cocks, T.M.; Jenkinson, D.H. Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature* 1979, 282, 415–417.

20. Hugues, M.; Romey, G.; Duval, D.; Vincent, J.P.; Lazdunski, M. Apamin as a selective blocker of the calcium-dependent potassium channel in neuroblastoma cells: Voltage-clamp and biochemical characterization of the toxin receptor. *Proc. Natl. Acad. Sci. USA* 1982, 79, 1308–1312.

21. Carbone, E.; Wanke, E.; Prestipino, G.; Possani, L.D.; Maelicke, A. Selective blockage of voltage-dependent K⁺ channels by a novel scorpion toxin. *Nature* 1982, 296, 90–91.

22. Miller, C.; Moczydlowski, E.; Latorre, R.; Phillips, M. Charybdotoxin, a protein inhibitor of single Ca²⁺-activated K⁺ channels from mammalian skeletal muscle. *Nature* 1985, 313, 316–318.

23. Judge, S.I.; Bever, C.T., Jr. Potassium channel blockers in multiple sclerosis: Neuronal Kv channels and effects of symptomatic treatment. *Pharmaco. Ther.* 2006, 111, 224–259.

24. Tytgat, J.; Chandy, K.G.; Garcia, M.L.; Gutman, G.A.; Martin-Eauclaire, M.F.; van der Walt, J.J.; Possani, L.D. A unified nomenclature for short-chain peptides isolated from scorpion venoms: α-KTx molecular subfamilies. *Trends Pharmacol. Sci.* 1999, 20, 444–447.

25. Jin, W.; Lu, Z. A novel high-affinity inhibitor for inward-rectifier K⁺ channels. *Biochemistry* 1998, 37, 13291–13299.

26. Mathie, A.; Veale, E.L. Therapeutic potential of neuronal two-pore domain potassium-channel modulators. *Curr. Opin. Investig. Drugs* 2007, 8, 555–562.

27. Swartz, K.J.; MacKinnon, R. Hanatoxin modifies the gating of a voltage-dependent K⁺ channel through multiple binding sites. *Neuron* 1997, 18, 665–673.

28. Swartz, K.J. Tarantula toxins interacting with voltage sensors in potassium channels. *Toxicon* 2007, 49, 213–230.

29. McManus, A.; Stefoski, D.; Rush, J. Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. *Ann. Neurol.* 1990, 27, 186–192.

30. Grissmer, S.; Nguyen, A.N.; Aiyar, J.; Hanson, D.C.; Mather, R.J.; Gutman, G.A.; Karmilowicz, M.J.; Auperin, D.D.; Chandy, K.G. Pharmacological characterization of five cloned voltage-gated K⁺ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. *Mol. Pharmacol.* 1994, 45, 1227–1234.

31. Garcia-Calvo, M.; Leonard, R.J.; Novick, J.; Stevens, S.P.; Schmalhofer, W.; Kaczorowski, G.J.; Garcia, M.L. Purification, characterization, and biosynthesis of margatoxin, a component of Centruroides margaritatus venom that selectively inhibits voltage-dependent potassium channels. *J. Biol. Chem.* 1993, 268, 18866–18874.

32. Aiyar, J.; Withka, J.M.; Rizzi, J.P.; Singleton, D.H.; Andrews, G.C.; Lin, W.; Boyd, J.; Hanson, D.C.; Simon, M.; Dethlefs, B.; et al. Topology of the pore-region of a K⁺ channel revealed by the NMR-derived structures of scorpion toxins. *Neuron* 1995, 15, 1169–1181.
36. Garcia, M.L.; Garcia-Calvo, M.; Hidalgo, P.; Lee, A.; MacKinnon, R. Purification and characterization of three inhibitors of voltage-dependent K⁺ channels from Leiurus quinquestriatus var. hebraeus venom. *Biochemistry* 1994, 33, 6834–6839.

37. Koschak, A.; Bugianesi, R.M.; Mitterdorfer, J.; Kaczorowski, G.J.; Garcia, M.L.; Knaus, H.G. Subunit composition of brain voltage-gated potassium channels determined by hongotoxin-1, a novel peptide derived from Centruroides limbatus venom. *J. Biol. Chem.* 1998, 273, 2639–2644.

38. Bagdany, M.; Batista, C.V.; Valdez-Cruz, N.A.; Somodi, S.; Rodriguez de la Vega, R.C.; Licea, A.F.; Varga, Z.; Gaspar, R.; Possani, L.D.; Panyi, G.; *et al*. Anuroctoxin, a new scorpion toxin of the α-KTx 6 subfamily, is highly selective for Kv1.3 over IKCa1 ion channels of human T lymphocytes. *Mol. Pharmacol.* 2005, 67, 1034–1044.

39. Mouhat, S.; Visan, V.; Ananthakrishnan, S.; Wulff, H.; Andreotti, N.; Grissmer, S.; Darbon, H.; de Waard, M.; Sabatier, J.M. K⁺ channel types targeted by synthetic OSK1, a toxin from Orthochirus scrobiculosus venom. *Biochem. J.* 2005, 385, 95–104.

40. Varga, Z.; Gurrola-Briones, G.; Papp, F.; Rodriguez de la Vega, R.C.; Pedraza-Alva, G.; Tajhya, R.B.; Gaspar, R.; Cardenas, L.; Rosenstein, Y.; Beeton, C.; *et al*. Vm24, a natural immunosuppressive peptide, potently and selectively blocks Kv1.3 potassium channels of human T cells. *Mol. Pharmacol.* 2012, 82, 372–382.

41. Han, S.; Yi, H.; Yin, S.J.; Chen, Z.Y.; Liu, H.; Cao, Z.J.; Wu, Y.L.; Li, W.X. Structural basis of a potent peptide inhibitor designed for Kv1.3 channel, a therapeutic target of autoimmune disease. *J. Biol. Chem.* 2008, 283, 19058–19065.

42. Han, S.; Hu, Y.; Zhang, R.; Yi, H.; Wei, J.; Wu, Y.; Cao, Z.; Li, W.; He, X. ImKTx88, a novel selective Kv1.3 channel blocker derived from the scorpion Isometrus maculates. *Toxicon* 2011, 57, 348–355.

43. Regaya, I.; Beeton, C.; Ferrat, G.; Andreotti, N.; Darbon, H.; de Waard, M.; Sabatier, J.M. Evidence for domain-specific recognition of SK and Kv channels by MTX and HsTx1 scorpion toxins. *J. Biol. Chem.* 2004, 279, 55690–55696.

44. Chi, V.; Pennington, M.W.; Norton, R.S.; Tarcha, E.J.; Londono, L.M.; Sims-Fahey, B.; Upadhyay, S.K.; Lakey, J.T.; Iadonato, S.; Wulff, H.; *et al*. Development of a sea anemone toxin as an immunomodulator for therapy of autoimmune diseases. *Toxicon* 2012, 59, 529–546.

45. Cuypers, E.; Abdel-Mottaleb, Y.; Koplij, I.; Rainier, J.D.; Raes, A.L.; Snyders, D.J.; Tytgat, J. Gambierol, a toxin produced by the dinoflagellate Gambierdiscus toxicus, is a potent blocker of voltage-gated potassium channels. *Toxicon* 2008, 51, 974–983.

46. Kauferstein, S.; Huys, I.; Lamthanh, H.; Stocklin, R.; Sotto, F.; Menez, A.; Tytgat, J.; Mebs, D. A novel conotoxin inhibiting vertebrate voltage-sensitive potassium channels. *Toxicon* 2003, 42, 43–52.

47. Chhabra, S.; Chang, S.C.; Nguyen, H.M.; Huq, R.; Tanner, M.R.; Londono, L.M.; Estrada, R.; Dhawan, V.; Chauhan, S.; Upadhyay, S.K.; *et al*. Kv1.3 channel-blocking immunomodulatory peptides from parasitic worms: Implications for autoimmune diseases. *FASEB J.* 2014, doi:10.1096/fj.14-251967.

48. Yang, W.; Feng, J.; Wang, B.; Cao, Z.; Li, W.; Wu, Y.; Chen, Z. BF9, the first functionally characterized snake toxin peptide with Kunitz-type protease and potassium channel inhibiting properties. *J. Biochem. Mol. Toxicol.* 2014, 28, 76–83.
49. Beeton, C.; Pennington, M.W.; Wulff, H.; Singh, S.; Nugent, D.; Crossley, G.; Khaytin, I.; Calabresi, P.A.; Chen, C.Y.; Gutman, G.A.; et al. Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channels for therapy of autoimmune diseases. *Mol. Pharmacol.* 2005, 67, 1369–1381.

50. Chandy, K.G.; Wulff, H.; Beeton, C.; Pennington, M.; Gutman, G.A.; Cahalan, M.D. K⁺ channels as targets for specific immunomodulation. *Trends Pharmacol. Sci.* 2004, 25, 280–289.

51. Lanigan, M.D.; Kalman, K.; Lefievre, Y.; Pennington, M.W.; Chandy, K.G.; Norton, R.S. Mutating a critical lysine in ShK toxin alters its binding configuration in the pore-vestibule region of the voltage-gated potassium channel, Kv1.3. *Biochemistry* 2002, 41, 11963–11971.

52. Liu, Q.H.; Fleischmann, B.K.; Hondowicz, B.; Maier, C.C.; Turka, L.A.; Yui, K.; Kotlikoff, M.I.; Wells, A.D.; Freedman, B.D. Modulation of Kv channel expression and function by TCR and costimulatory signals during peripheral CD4⁺ lymphocyte differentiation. *J. Exp. Med.* 2002, 196, 897–909.

53. Grissmer, S.; Lewis, R.S.; Cahalan, M.D. Ca²⁺-activated K⁺ channels in human leukemic T cells. *J. Gen. Physiol.* 1992, 99, 63–84.

54. Beeton, C.; Wulff, H.; Barbaria, J.; Clot-Faybesse, O.; Pennington, M.; Bernard, D.; Cahalan, M.D.; Chandy, K.G.; Beraud, E. Selective blockade of T lymphocyte K⁺ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 2001, 98, 13942–13947.

55. Deshmukh, V.A.; Tardif, V.; Lyssiotis, C.A.; Green, C.C.; Kerman, B.; Kim, H.J.; Padmanabhan, K.; Swoboda, J.G.; Ahmad, I.; Kondo, T.; et al. A regenerative approach to the treatment of multiple sclerosis. *Nature* 2013, 502, 327–332.

56. Fazakerley, J.K.; Walker, R. Virus demyelination. *J. Neurovirol.* 2003, 9, 148–164.

57. Steinman, L.; Zamvil, S.S. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. *Ann. Neurol.* 2006, 60, 12–21.

58. Baker, D.; Gerritsen, W.; Rundle, J.; Amor, S. Critical appraisal of animal models of multiple sclerosis. *Mult. Scler.* 2011, 17, 647–657.

59. Breland, A.E.; Currier, R.D. Scorpion venom and multiple sclerosis. *Lancet* 1983, 2, p. 1021, doi:10.1016/S0140-6736(83)90996-0.

60. Matheu, M.P.; Beeton, C.; Garcia, A.; Chi, V.; Rangaraju, S.; Safrina, O.; Monaghan, K.; Uemura, M.I.; Li, D.; Pal, S.; et al. Imaging of effector memory T cells during a delayed-type hypersensitivity reaction and suppression by Kv1.3 channel block. *Immunity* 2008, 29, 602–614.

61. Chiu, S.Y.; Zhou, L.; Zhang, C.L.; Messing, A. Analysis of potassium channel functions in mammalian axons by gene knockouts. *J. Neurocytol.* 1999, 28, 349–364.

62. Rasband, M.N.; Trimmer, J.S.; Schwarz, T.L.; Levinson, S.R.; Ellisman, M.H.; Schachner, M.; Shrager, P. Potassium channel distribution, clustering, and function in remyelinating rat axons. *J. Neurosci.* 1998, 18, 36–47.

63. Beeton, C.; Wulff, H.; Standifer, N.E.; Azam, P.; Mullen, K.M.; Pennington, M.W.; Kolski-Andrea, A.; Wei, E.; Grino, A.; Counts, D.R.; et al. Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases. *Proc. Natl. Acad. Sci. USA* 2006, 103, 17414–17419.

64. Hultqvist, M.; Olofsson, P.; Gelderman, K.A.; Holmberg, J.; Holmdahl, R. A new arthritis therapy with oxidative burst inducers. *PLoS Med.* 2006, 3, e348.
65. Toldi, G.; Bajnok, A.; Dobi, D.; Kaposi, A.; Kovacs, L.; Vasarhelyi, B.; Balog, A. The effects of Kv1.3 and IKCa1 potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis. *Immunobiology* 2013, 218, 311–316.

66. Schmitz, A.; Sankaranarayanan, A.; Azam, P.; Schmidt-Lassen, K.; Homerick, D.; Hansel, W.; Wulff, H. Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases. *Mol. Pharmacol.* 2005, 68, 1254–1270.

67. Wulff, H.; Pennington, M. Targeting effector memory T-cells with Kv1.3 blockers. *Curr. Opin. Drug Discov. Dev.* 2007, 10, 438–445.

68. Toldi, G.; Vasarhelyi, B.; Kaposi, A.; Meszaros, G.; Panczel, P.; Hosszufalusi, N.; Tulassay, T.; Treszl, A. Lymphocyte activation in type 1 diabetes mellitus: The increased significance of Kv1.3 potassium channels. *Immunol. Lett.* 2010, 133, 35–41.

69. Couser, W.G.; Johnson, R.J. The etiology of glomerulonephritis: Roles of infection and autoimmunity. *Kidney Int.* 2014, 86, 905–914.

70. Hyodo, T.; Oda, T.; Kikuchi, Y.; Higashi, K.; Kushiyama, T.; Yamamoto, K.; Yamada, M.; Suzuki, S.; Hokari, R.; Kinoshita, M.; et al. Voltage-gated potassium channel Kv1.3 blocker as a potential treatment for rat anti-glomerular basement membrane glomerulonephritis. *Am. J. Physiol. Renal Physiol.* 2010, 299, F1258–F1269.

71. Nguyen, W.; Howard, B.L.; Neale, D.S.; Thompson, P.E.; White, P.J.; Wulff, H.; Manallack, D.T. Use of Kv1.3 blockers for inflammatory skin conditions. *Curr. Med. Chem.* 2010, 17, 2882–2896.

72. Kundu-Raychaudhuri, S.; Chen, Y.J.; Wulff, H.; Raychaudhuri, S.P. Kv1.3 in psoriatic disease: PAP-1, a small molecule inhibitor of Kv1.3 is effective in the SCID mouse psoriasis—Xenograft model. *J. Autoimmun.* 2014, 55, 63–72.

73. Gilhar, A.; Bergman, R.; Assay, B.; Ullmann, Y.; Etzioni, A. The beneficial effect of blocking Kv1.3 in the psoriasiform SCID mouse model. *J. Investig. Dermatol.* 2011, 131, 118–124.

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