Introduction

Autoimmune diseases are common maladies caused by an anomalous function of the immune system. Pathological activation results in immune cells targeting cellular or organ-specific self-antigens. The disease usually progresses to local or even systemic inflammation processes which seriously compromise the homeostasis of the tissue, as well as its function [1]. The consequences of autoimmune diseases are variable as more than 80 have been reported in humans [2].

Some autoimmune diseases, such as multiple sclerosis, RA or myasthenia gravis, cause highly debilitating effects on the wellbeing of the patients [3–5]. Other maladies, such as type 1 diabetes mellitus or celiac disease are equally dependent on pharmacological control [6,7]. Regardless, the total prevalence of autoimmune diseases is up to 4.5% in the United States [8]. Therefore, autoimmune diseases do not just threaten the wellbeing of millions of people worldwide but also present a high socioeconomic impact.

Autoimmune diseases do not typically trigger acute illnesses; however, their symptoms appear as an accumulation of hidden insults to certain tissues in a chronic manner [9]. During development, T and B lymphocytes reactive to self-antigens can be produced. These cells tend to be wiped out or functionally silenced early on by a process of overactivation (tolerance) [10]. Nevertheless, these mechanisms fail in diverse ways, generating lymphocytes that should not be reactive.

Several theories claim differential onsets for this event. The cryptic determinant theory states that some antigens or antigen determinants are usually hidden from immune detection [11]. This could be because an antigen is in a remote location (e.g., the eye) or because the typical conformation of a protein normally hides the antigenic determinant. The inaccessibility of the antigen results in self-antigen-reactive lymphocytes, which are not correctly silenced. Other theories support foreign antigens mimicking self-antigens [12], which cause cross-reactivity from the lymphocytes and alterations of the glycosylation profile of the immune system components [13]. The most controversial hypothesis, though, links the high penetrance of autoimmune diseases in developed countries with a higher level of hygiene [14].
Theoretically, improved hygiene results in lymphocytes being exposed to fewer antigens and are, therefore, overreactive to self-antigens. Neither of these theories has been proven to be the main cause of autoimmune diseases, which suggests that it may be a combination of all of them. Additionally, the existence of familial forms of these diseases and differences between the familial and sporadic forms reveals a genetic implication for pathogenicity [15–17]. Therefore, the pathophysiology of autoimmune diseases shows a complexity comparable to those of cancer processes.

In autoimmune diseases, the voltage-dependent potassium channel Kv1.3 plays a paramount role. T helper lymphocytes activate in contact with an APC (antigen presenting cell), such as macrophages or B lymphocytes (Fig. 1). APC presents the antigen using MHC (major histocompatibility complex)/TCR recognition initiates a signal transduction cascade, which implies the activation of phospholipase Cγ (PLC). PLC activates diacylglycerol (DAG) and 1,4,5-inositol trisphosphate (IP3) triggering the depletion of internal ER (endoplasmic reticulum) Ca2+ stores. The intracellular Ca2+ augmentation activates calcium release-activated channels (CRAC), which drives more Ca2+ into the cell producing depolarization. Kv1.3 opens by depolarization causing K+ efflux that hyperpolarizes the membrane. In addition, the rise in internal Ca2+ concentration [Ca2+]i activates the intermediate-conductance Ca2+-activated K+ channel (Kc3.1). The synergy of the two K+ channels keeps a negative membrane potential adequate for a sustained Ca2+ influx through CRAC channels. Finally, a sustained Ca2+ signal activate nuclear processes, such as gene remodeling and/or proliferation. An increase of Kv1.3 abundance elevates the response increasing the aggressiveness of the T cell.

Potential Kv1.3 therapies could target directly either the channel or putative associations and spatial locations. The Kv1.3 channelosome is a functional complex that fine-tunes the Kv1.3 activity. Kv1.5, exhibiting differential traffic, targeting and electrophysiological and pharmacological characteristics, heterotetramerizes with Kv1.3. By altering the stoichiometry of the heteromer, the properties of the functional complex differ. KCNE4, which negatively modulates the activity and the surface expression of KCNE4, could be contemplated as a therapeutical target. The Matrix metalloproteinase-23 also interacts with Kv1.3 retaining the channel at the ER. Animal venoms (toxins) and plant derivatives (psoralsens) are quite specific and potent Kv1.3 blockers. Miscellaneous compounds, such as viral molecules (VP1), defensins and antifungals, also show inhibitory activity. Diclofenac, a nonsteroidal anti-inflammatory drug (NSAIDs), impairs the Kv1.3 gene expression. Finally, during the immune system response, Kv1.3 localizes at the immunological synapse between APC and T cell. This synapse concentrates lipid rafts. Controlling this spatial localization, by disrupting rafts with methyl-beta-cyclodextrin, impairs the activity of Kv1.3.

See text for further details.
signal results in the production of IP₃ (inositol triphosphate) and DAG (diacylglycerol). At the same time, these molecules spark signaling pathways which elevate the intracellular Ca²⁺ concentration activating Ca²⁺-dependent Kᵥ₃.1 channels and Kᵥ1.3 [18,19]. Both channels contribute to maintain the Ca²⁺ influx, which triggers gene remodeling, ending in activation and clonal expansion of lymphocytes (Fig. 1). These channels are essential for the immune activation. While Kᵥ₃.1 dominates the immature preactivated T cells, Kᵥ1.3 is the most important actor in the mature T cells [18,20,21]. T EM cells are mature T lymphocytes which have been exposed repetitively to their antigen. These cells are usually present in peripheral tissues and in circulating blood and lymph [22]. Because T EM cells usually reside in inflamed tissues, the effect is very fast facilitating their action in autoimmune diseases once they start to proliferate. Indeed, T EM cells with high Kᵥ1.3 expression are found in areas of inflammation related to autoimmune diseases [23]. Therefore, T EM and Kᵥ1.3 could be responsible for the chronicity of autoimmune diseases [19,24]. Evidence demonstrates that Kᵥ1.3-inhibiting drugs also significantly improve the symptoms of autoimmune diseases [25-27].

RA is an autoimmune disorder that mainly targets the synovial joints [28]. Unlike other autoimmune diseases, the trigger for RA is unknown, which hinders its diagnosis and prognosis. Furthermore, several different phenotypes exist, which confirms the heterogeneity of the disease [29]. Even though the pathogenesis is not well understood, RA follows the same pattern as other autoimmune diseases: inflammation, hyperplasia (by swelling) and destruction of the affected tissue, self-antibody production and some systemic effects [28]. The disease focuses on the synovial joints and typical symptoms are synovial inflammation, which progresses to the cartilage, and bone destruction. Because the joint is a structural part of the body, this also implies a certain grade of progressive deformity [28]. In this context, the rheumatoid factor, tightly linked to the disease, is reactive against the Fc portion of the IgG [30]. This interaction provokes a plethora of systemic disorders, such as cardiovascular or pulmonary symptoms, as well as fever [28]. Therefore, RA gradually impairs the motility of patients, which affects them psychologically. This scenario creates a positive feedback loop, which further aggravates the other symptoms [31].

Despite the diversity of autoimmune diseases as well as their chronic and incapacitating nature, the present treatments are limited and suboptimal. The existing first-line medication to treat RA and other autoimmune diseases is methotrexate [32,33], as well as other DMARD (Disease-modifying antirheumatic drugs) such as hydroxychloroquine. Methotrexate is an antimetabolite for the folate molecule which binds to DHFR (dihydrofolate reductase), an enzyme which catalyzes the reaction of dihydrofolate to tetrahydrofolate [34,35]. Because this step is necessary for nucleic bases biosynthesis and amino acid processing, methotrexate inhibits the synthesis of DNA, RNA and proteins. Therefore, the antirheumatic function is not selectively targeted but similar to chemotherapy, a collateral side effect of toxicity. Indeed, methotrexate was previously used for the treatment of cancer [36,37]. Although the dose of methotrexate used in RA is lower than the dose used for oncology, long treatment durations often result in adverse effects, such as hair loss, nausea or headaches [38]. Furthermore, methotrexate is a long-known teratogen and abortifacient, which complicates the combination of pregnancy and the disease [39]. Despite these side effects, methotrexate has continued to serve as an essential medicine [40].

Other medications used for the treatment of RA depend on the severity of symptoms and the course of the disease [41]. NSAIDs (Non-steroidal anti-inflammatory drugs), such as ibuprofen, do help in relieving pain and reducing inflammation. However, a sustained intake can result in gastric damage and compromise cardiovascular safety. In addition, steroids like prednisone have a great anti-inflammatory effect but high doses provoke side effects such as high glycaemia or fluid retention [42]. Finally, biologic agents, also known as biologic response modifiers, have been developed. However, biologic agents, targeting the immune system, compromise the immune response elevating the risk of cancer or infections, such as tuberculosis [43,44]. The actual choice is DMARD, combined with NSAIDs and steroids, to control pain and inflammation. Biologic agents are only considered once DMARDs fail [45].

Above mentioned therapies are effective to some degree, slowing the course of the disease, but triggering side effects. Therefore, development of new antirheumatic compounds with better therapeutic window and target effectivity deserves more attention.

2. Kᵥ1.3 as a therapeutic target for autoimmune diseases

Kᵥ1.3 is a voltage-gated potassium channel which opens at depolarizing membrane potentials [46]. The channel is present in different regions of the CNS (central nervous system), such as the olfactory bulb, and in some nonexcitable tissues, such as adipose tissue or kidneys [47-49]. Regardless, the most essential role of Kᵥ1.3 resides in the immune system. Kᵥ1.3 is expressed in lymphocytes. Kᵥ1.3 is present in the plasma membrane, and targets to the immune synapse during lymphocyte activation [50], but is also detectable in different sub-cellular localizations, such as cis-Golgi, nuclear membrane and mitochondria. However, this review will deal mainly with Kᵥ1.3 located at the cell surface. T lymphocytes have their TCRs (T-cell receptors) clustered in specific membrane microdomains, called lipid rafts, enriched in cholesterol [51]. The concentration of Kᵥ1.3 in these regions facilitates lymphocyte activation upon an antigen presentation by APCs [52]. In fact, Kᵥ1.3 function is highly dependent on lipid raft location.
In fact, the manipulation of membrane lipid composition alters the voltage- and time-dependent gating of the channel [54].

Kv1.3 function is predominant in lymphocyte physiology. Therefore, a connection between Kv1.3 and immune physiology is not surprising. NSAIDs, such as diclofenac, as well as some glucocorticoids, like dexamethasone, reduce Kv1.3 activity in immune cells [55–57]. Moreover, inhibition of Kv1.3 activity impairs the immune response [58]. Thus, pharmacological blockade of Kv1.3 inhibits the proliferation and cytokine production of the pathogenic T<sub>EM</sub> cells while sparing other lymphocyte subpopulations [59]. During the last decade, Kv1.3 blockers have been developed and show a promising effect on autoimmune diseases [60].

In addition to lymphocytes, Kv1.3 is also a predominant channel in the APC of the macrophage lineage [61], also governing cell activation. Furthermore, Kv1.3 abundance correlates with the activation of macrophages. Thus, LPS (lipopolysaccharide)-dependent activation of macrophages increases Kv1.3 expression, while glucocorticoids, such as dexamethasone, reduces the amount of Kv1.3 [55]. In this scenario, macrophages undergo a molecular remodeling of Kv isoforms, such as Kv1.5, which heterotetramerizing with Kv1.3 modulates the channel [62]. When Kv1.3 increases, the stoichiometry of functional tetramers favors Kv1.3 and results in channels with molecular properties similar to Kv1.3. In contrast, a decrease in Kv1.3 levels results in heteromers with more Kv1.5 units, recapitulating properties of the Kv1.5 homotetramer [61,62]. In this context, Kv1.5 exhibits less membrane expression and activity, and cells are also less sensitive to physiological membrane potentials, which results in a higher threshold of APC activation. In addition, Kv1.3/Kv1.5 heterotetramers are reluctant to Kv1.3 blockers, such as margatoxin which hinders pharmacological treatments [63]. In this context, only pсорa-4, display similar potency for heteromers Kv1.3/Kv1.5 [48].

Kv1.3 also interacts with the regulatory subunit KCNE4, impairing both membrane localization and activity [64,65]. Kv1.3 has notorious membrane expression partly due to an anterograde signature (YMVIEE) located in the C-terminus [66]. This signature interacts with Sec24 and facilitates the forward traffic of Kv1.3 via the COPII machinery. KCNE4 association, masking this signal, impairs the COPII-mediated anterograde transport and the expression of the channel at the plasma membrane [67]. Similar to Kv1.3, KCNE4 is tightly regulated in leukocytes under several insults, which could fine-tune the channel function [64,65]. Therefore, KCNE4 should be considered as a potential target, but further research must be conducted.

Kv1.3 functional activity is modulated by a wide repertoire of ancillary peptides and signaling pathways. For example, Na<sub>a</sub>B1, a regulatory subunit for Na<sub>a</sub> channels, also modifies Kv1.3 gating by presumably strengthening the coupling between activation of the voltage-sensing domain of the channel and the opening of the pore [68]. In addition, antifungal compounds, such as nystatin and amphotericin B, exert a potent inhibition of Kv1.3 from the cytoplasmic side [69]. However, defensin peptides, such as plectasin, block the channel from the extracellular side [70,71]. Sphingomyelin also favors a more electropositive threshold of activation in T-lymphocytes [72]. Therefore, these molecules reduce the Kv1.3 aperture within the same voltages. Furthermore, the pro-domain of MMP23 (matrix metalloproteinase 23) forces the intracellular retention of Kv1.3, thereby depleting the membrane of channels [73]. Interestingly, MMP23 is highly expressed in some subtypes of lymphocytes, such as the NK (natural killer) cells [74]. Finally, components of some viruses, such as the capsid protein VP1 of Parvovirus B19, downregulate Kv1.3, as well as other ion channels [75].

As mentioned above, Kv1.3 can be modulated by several agents which use completely different mechanisms. Kv1.3-based therapies are advantageous because unlike other Kv channels, Kv1.3 has minor implications in heart physiology. Thus, one of the most limiting factors in ion channel drug development, which is the modification of heart physiology, should not hinder Kv1.3 blockers [76]. Furthermore, the Kv1.3 knockout (KO) animal model is viable [77]. Kv1.3 gene deletion surprisingly does not trigger a massive effect on the immune system likely because of a compensatory increase in Cl<sup>-</sup> currents in lymphocytes. However, Kv1.3 KO mice show a decrease in the severity of autoimmune diseases, as well as a minor prevalence of tissue-specific T<sub>EM</sub> cells [78]. Furthermore, the deficiency of Kv1.3 favors the proliferation of T lymphocytes with suppressive properties instead of T<sub>EM</sub> [79]. These results correlate with several in vivo studies that have demonstrated that genetic ablation or pharmacological inhibition of Kv1.3 greatly diminishes the production of proinflammatory cytokines from microglia in an LPS-directed model [80]. This evidence justifies targeting macrophage, in addition to lymphocytes for the treatment of autoimmune diseases.

Recent work demonstrated that patients with RA also tend to present comorbidities such as diabetes (∼15%), hypertension (∼40%), depression (∼15%) and gastroduodenal ulcer (∼11%) [81]. Whether these maladies are related to RA is an open debate not covered in this review. RA patients exhibit an increased risk of suffering from certain illnesses; therefore, these maladies should be screened, diagnosed and treated when positive.

Surprisingly, the scope of Kv1.3 function is wider than initially expected. Thus, blockade of Kv1.3 not only ameliorates the prognosis in autoimmune disease models [27,82] but also shows promising results for other physiological processes. The Kv1.3 KO mouse exhibited enhanced odorant ability (thus, the nickname super-smeller mouse) and resistance to diet-induced obesity [83,84]. These effects could be a consequence of increases in physical activity and basal metabolic rate, which depend on the olfactory bulb. In wild type animals, a similar effect is triggered by the activation of the insulin receptor in the olfactory bulb, which inhibits Kv1.3 by phosphorylation. Therefore, insulin resistance hinders this process, which explains the positive feedback of symptoms in type 2 diabetes mellitus. Furthermore, insulin resistance normally includes high adiposity, anxiety, and reduction in cognitive abilities [85]. Interestingly, insulin resistance (diabetes) and anxiety (depression) are tightly related with some comorbidities of RA. In this context, some works corroborate that the genetic ablation or inhibition of Kv1.3 improves mood and associative learning in rodents, aside from triggering resistance to diet-induced obesity [83,86]. Moreover, proof-of-concept studies have demonstrated the potential use of Kv1.3 blockers to treat other neurological syndromes such as Alzheimer [87]. Therefore, pharmacological blockade of Kv1.3 activity could be beneficial for RA as well as some related disorders.

3. Kv1.3 in rheumatoid arthritis: the other side of the coin

Kv1.3 stands out as a potential target for the treatment of RA among the autoimmune disorders. However, controversial results arguing against Kv1.3 have opened the debate.

First, the super-smeller mouse Kv1.3 KO model exhibits minor immune defects [77]. As mentioned above, the reason could be remodeling of the Cl<sup>-</sup> currents, which could compensate for the deficiency in K<sup>+</sup> currents in T lymphocytes. Additionally, murine lymphocytes possess additional K<sup>+</sup> channels and transporters that could drive K<sup>+</sup> in the absence of Kv1.3 [88–90]. Such a repertoire of channels generates a great variety of ion channel phenotypes in these cells that could mask the importance of Kv1.3 in rodents. In contrast, human T lymphocytes mainly express Kv1.3, with relatively minor influence from other subunits. Another source of variability is that the human Kv1.3 has an additional 5′ upstream starting codon, which produces a 52-residue longer N-terminal domain. This sequence seems to impair the surface membrane expression of the channel in Xenopus oocytes [68]. This longer domain could trigger differential phenotypes in human T lymphocytes. No pharmacological studies have been conducted on the longer human Kv1.3 form and Kv1.3 blockers, validated in rodents, could trigger unexpected behaviors in humans. Therefore, additional experiments should be conducted on the longer Kv1.3 human form as
well as with the primate, pig or fish orthologues, which also possess the additional N-terminal sequence [68]. Finally, additional features for the therapeutic use of Kv1.3 in RA are the non-conducting properties of the channels [91]. In this context, Kv1.3 regulates cell proliferation in the vascular tissue by an ion-flux independent mechanism [92].

4. Pharmacological development

In this context, new research aims to develop Kv1.3 blockers, which could be used in the treatment of autoimmune diseases. Kv1.3 is affected by a great collection of natural toxins. Therefore, research has focused on modification, thereby improving the specificity, pharmacokinetics and pharmacodynamics [82,93]. The most well-known natural toxins come from animal venoms, such as scorpions (margatoxin and kalitoxin) and anemones (ShK) [86,94,95]. Other blockers are mainly small molecules (PAP-1, Psora-4, and clofazimine), mostly from plant origin [96,97].

One promising molecule is dalazatide, a modified form of ShK (Stichodactyla toxis) [98]. ShK is a 35-residue peptide produced by the sea anemone Stichodactyla helianthus, which blocks neuronal and lymphocyte voltage-dependent potassium channels [95]. Because ShK exhibits affinity towards Kv1.3 but also Kv1.1 and Kv1.6 [99], several ShK analogs were developed. For instance, ShK-186 increased the selectivity for Kv1.3 by modifying some amino acids of the structure [100]. Thus, ShK-186, renamed dalazatide, became the first Kv1.3 blocker to be tested on humans [98]. The primary scope for this medication is psoriasis, an autoimmune disease mainly targeting the skin, currently undergoing phase II clinical trials. However, recent results also support the use of dalazatide in other autoimmune diseases such as sporadic inclusion body myositis and lupus nephritis [101,102]. The similar etiology and molecular background of these autoimmune diseases may also broaden the scope of the medication, as changes in the formulation can facilitate use in many different diseases. The pharmacokinetics of dalazatide support lasting treatments, as the medication (ClinicalTrials.gov: NCT02435342) shows little to no accumulation after 29 days [98]. Moreover, dalazatide administration is subcutaneous [98]. In this context, alternative administration routes are being considered by several groups. For instance, chitosan-based gels have been proven to improve the buccal mucosal absorption of ShK [103].

Even though dalazatide is a first-in-class medication using Kv1.3 as a target against autoimmune diseases, many advances are continuously advancing this field. Scorpion AnTx (anurotoxin) has been modified to reduce its affinity towards Kv1.2 in favor of Kv1.3 [104], and scorpion Vm24, OsK1 and OdK2 have been fused with an antibody Fc (constant fraction) to increase their plasma half-life [105,106]. Pulmonary delivery has also been studied as a noninvasive alternative and has already been successfully demonstrated with an HsTx1 (Heterometrus spinifer toxin) analog [107].

In parallel, Kv1.3 modulation should not be limited to a blockade (Fig. 2). As mentioned above, Kv1.3 heteromerizes with other isoforms, such as Kvβ5; and regulatory subunits such as KCNE4, and the Kvβ family [64,108,109] in native cells. Interaction with such subunits modifies the activity and threshold of activation of the complex, thus altering the leukocyte activation [62,63,67]. Efforts to control the expression of such proteins or to emulate their effects would be highly advisable to modulate the channel with a less binary outcome than blockers.

Therefore, the use of Kv1.3 as a therapeutic target for autoimmune diseases in general and RA in particular is a novel method with huge potential. Combinational therapies for RA deserve further attention [110,111]. Specific Kv1.3 blockers could substitute actual medications, such as NSAIDs or steroids, which partially targeting Kv1.3, exhibit harmful side effects [55–57].

Finally, because most autoimmune diseases share an elevated TEM population, which tightly depends on Kv1.3, Kv1.3-based therapies could be repurposed and extended further than RA [18,20,101,102].

5. Concluding remarks

RA is an autoimmune disease with serious effects on the wellbeing of patients and social environments. The progression of symptoms greatly impairs the normal life of patients both physically and psychologically. Therefore, the socioeconomic cost of this disease is very high for the health system. Considering that RA etiology shares most features of many autoimmune diseases, the need for an effective treatment deserves more effort.

The current first-line treatment for RA, methotrexate, is effective but carries potential diverse side effects similar to chemotherapy drugs. These harmful consequences sometimes lead to a certain level of treatment discontinuation. In persistent and long-term chronic diseases, the adherence to the treatment may have a marked difference on the wellbeing of the patient. Moreover, as methotrexate is a teratogen, a pregnancy-friendly treatment could help female patients improve their quality of life by preventing the disease from affecting their family planning.

In this context, Kv1.3 is a promising therapeutic target for the treatment of RA and for autoimmune diseases in general (Table 1). Kv1.3 plays a relevant role during immune system responses. This role is even more apparent in the TEM cells, which are the main driving force of autoimmune progression. Consequently, the inhibition of Kv1.3 ameliorates autoimmune disease symptoms in animals. Moreover, Kv1.3 stands out as a more specific alternative to treatments such as methotrexate because both the pharmacological inhibition and genetic ablation of the channel in animal models result in no pernicious effects on the animals. Nonetheless, differences between rodent and human lymphocytes are not irrelevant. Human lymphocytes express mainly Kv1.3 as a unique voltage-gated potassium channel, while rodents also possess additional Kv1 isoforms. Additionally, human Kv1.3 possesses an additional upstream methionine codon, which adds 52 amino acids to the protein. These differences could add variability to the development of Kv1.3 blockers and should be considered when transitioning newly developed drugs to the clinical stage.

Taking into account all the limitations mentioned throughout this review, a great amount of resources is being funneled into both basic and clinical research for Kv1.3 as a potential therapeutic agent in several autoimmune diseases including RA.

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