Ion channels and anti-cancer immunity

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The outcome of a malignant disease depends on the efficacy of the immune system to destroy cancer cells. Key steps in this process, for example the generation of a proper Ca²⁺ signal induced by recognition of a specific antigen, are regulated by various ion channel including voltage-gated Kv1.3 and Ca²⁺-activated KCa3.1 K⁺ channels, and the interplay between Orai and STIM to produce the Ca²⁺-release-activated Ca²⁺ (CRAC) current required for T-cell proliferation and function. Understanding the immune cell subset-specific expression of ion channels along with their particular function in a given cell type, and the role of cancer tissue-dependent factors in the regulation of operation of these ion channels are emerging questions to be addressed in the fight against cancer disease. Answering these questions might lead to a better understanding of the immunosuppression phenomenon in cancer tissue and the development of drugs aimed at skewing the distribution of immune cell types towards killing of the tumour cells.

1. Introduction

Cancer is a complex disease in which the uncontrolled proliferation of malignantly transformed cells constitutes the major pathological picture. Accumulated data indicate that cancer cells cannot be studied in isolation, but should be investigated together with the surrounding ‘tumour microenvironment’ (TM) [1]. TM is formed by mesenchymal, endothelial and immune cells enmeshed in a network of extracellular matrix (ECM) proteins and soluble factors. The TM strongly contributes to tumour progression and influences different aspects of tumour cell behaviour via a variety of interactions including cell-to-cell or cell-to-matrix signals. Paul Ehrlich’s recognition in 1909 of the importance of the host immune response to tumours led to the ‘cancer immunosurveillance’ [2] hypothesis and its refinement, the ‘cancer immunoediting theory’ [3], whereby cancer cells and immune cells engage in a dynamic process of elimination, equilibrium and escape (reviewed in [4]). The elimination phase is characterized by a concerted interplay between innate and adaptive immunity through which developing tumours are eradicated on the basis of their expression of specific antigens. During the equilibrium phase, adaptive immune responses impose immune-selection pressure on tumour cells resulting in clones with reduced immunogenicity. Thus, even in the presence of continuous eradication, tumour cells resistant to killing by the immune system are generated. Probably the most complex interaction between tumour cells and the immune system occurs during the escape phase, whereby the immune response fails to completely eliminate the tumour cancer cells that can resist, avoid or suppress the anti-tumour immune response are selected, leading to tumour-escape and a progressively growing tumour. In addition to the reduction in immunogenicity of tumour cells and expression of anti-apoptotic molecules, a key contributor to escape is the development of a complex immunosuppressive network in the TM. Production of growth factors (e.g. TGF-β), interleukins (e.g. IL-10) and other soluble immunosuppressive cytokines derived from immune and tumour cells, and the recruitment of immunosuppressive cells such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) contribute to the failure of the immune system in controlling tumour growth [5,6]. In this
review, we discuss the network of ion channels in immune cells, their role in regulating the adaptive and innate responses, and the potential therapeutic value of specific channel blockers as immune modulators.

2. Physiological functions of ion channels in immune cells

Even though cellular components of the immune system are classically non-excitatory cells, i.e. they do not show action potential upon stimulation, ion channels still play a fundamental role in the function of lymphocytes, macrophages, monocytes and dendritic cells, both under physiological and pathophysiological conditions. The properties and functions of these channels have been reviewed extensively recently [7,8], therefore we give a short summary of this subject below and focus on ion channels and their expression relevant to the topic of the review.

(a) Ion channels in lymphocytes

(i) K⁺ channels in lymphocytes

Lymphocytes express predominantly two K⁺ channels, the voltage-gated Kv1.3 (encoded by the KCNA3 gene) and the calcium-activated K⁺ channels (also known as IKCa1; encoded by the KCNN4 gene). Kv1.3 and KCa3.1 channels are similar regarding the conductance properties (both channels are highly K⁺ selective and have similar single-channel conductance in the order of 10–14 pS), however they are remarkably different in their gating and blocker sensitivity [7,9]. Kv1.3 channels are activated by depolarization of the membrane, the activation threshold of the channel is close to the resting potential of the T cells (approx. −50 mV), the current is quickly activated and then inactivated by the slow P/C-type mechanism. On the contrary, KCa3.1 channels are solely activated by the increase in the cytosolic free Ca²⁺ concentration, and they are deactivated even after prolonged exposure to increased cytosolic Ca²⁺ concentration. The activation threshold for the KCa3.1 channels is approximately 200 nM, the calcium sensor is calmodulin associated permanently with the C-terminus of the channel. Besides the differences in gating, significant differences in the blocker sensitivity of Kv1.3 and KCa3.1 allow the activity of these channels to be modulated by organic small molecules and peptide toxin inhibitors (see below). The contribution of Kv1.3 and KCa3.1 channels to the regulation of the membrane potential in T cells is determined by their relative expression depending on the T cells subtype [10]. As a consequence, the inhibition of either Kv1.3 or KCa3.1 by the appropriate selective blockers can inhibit cell proliferation and function in a T-cell-subtype-specific manner, as discussed later in this review. In addition to Kv1.3, other K⁺ channels, for example two-pore domain containing K⁺ channels (K2Ps, such as TASK1-TASK3 [11,12]), have been suggested to contribute to membrane potential regulation of T cells.

(ii) Ca²⁺ channels in lymphocytes

Engagement of immunoreceptors, such as T-cell and B-cell antigen receptors, causes the release of Ca²⁺ from the endoplasmic reticulum (ER) Ca²⁺ stores as a result of binding of inositol trisphosphate (IP₃) to IP₃ receptors. The dominant pathway of intracellular Ca²⁺ increase in both T and B lymphocytes, however, is through the calcium release-activated Ca²⁺ (CRAC) channels. Stromal interaction proteins, STIM1 and STIM2, monitor the ER Ca²⁺ stores through EF-hand motifs, and upon depletion of the store STIM proteins are activated, oligomerized and redistributed into discrete puncta located in junctional ER sites that are in close proximity to the plasma membrane [8,13]. Direct binding of a cytoplasmic domain of STIM1 to the N- and C-termini of ORAI1, the pore-forming subunit of the CRAC channel, results in the formation of the functional CRAC channel. The CRAC channel displays high Ca²⁺ selectivity and extremely small single-channel conductance [14]. The activity of CRAC is not required for T-cell development as KO and transgenic mice and patients deficient in STIM1 and/or ORAI1 have normal numbers of T lymphocytes [15]. However, the function of these cells is drastically reduced and patients exhibit a severe combined immunodeficiency.

In addition to the major Ca²⁺ entry pathway provided by the CRAC channels, several alternative pathways have been suggested to contribute to calcium entry into T cells [8]. One of these is the store-operated Ca²⁺ entry (SOCE) mediated by TRP family channels [16]. Historically, TRPC channels were first suggested to be responsible for the CRAC current but later it turned out that CRAC channels are highly selective for Ca²⁺ as opposed the cation non-selective nature of TRP channels [16]. The mechanism of activation of TRPC channels is very controversial. Data strongly suggest that they are activated by mechanisms dependent on phospholipase C, but whether store-depletion and interaction with the ER Ca²⁺ sensor STIM is involved or not is currently debated [17,18]. Nevertheless, TRP-mediated Ca²⁺ influx seems to be important for lymphocyte function as, for example, TRPC3 modulates Ca²⁺-dependent proliferation of primary CD4⁺ T cells under reduced (limiting) extracellular Ca²⁺ conditions [19]. Alternatives Ca²⁺ entry mechanisms also include the P2X-type ionotropic purinergic receptors and voltage-gated Ca²⁺ channels, although the functional existence of the latter channels has not been demonstrated [7].

(iii) Role of ion channels in the antigen-dependent activation and proliferation of T cells

Antigen presentation evokes a sequence of events leading to the activation and proliferation of the antigen-specific T cells. During the initial antigen recognition step, the T-cell receptor/CD3 complex coalesces at an immunological synapse (IS) together with kinases/phosphatases. In the IS, key receptors and signalling molecules are organized to provide efficient signal transduction and termination (reviewed in [20]). The mitogenic signal emanating from the IS induces the breakdown of PIP₂ to IP₃, and the binding of IP₃ to the IP₃ receptor causes the release of Ca²⁺ from the store followed by the activation of the CRAC channels and the consequent Ca²⁺ influx. The sustained Ca²⁺ influx activates the calcineurin-NFAT pathway leading to new gene transcription. Deletion or mutations of STIM1 or ORAI1 in human T cells abolishes the Ca²⁺ influx, thereby leading to a failure of proliferation in response to TCR- or mitogen-dependent proliferation and defective production of many cytokines, including interleukin–2 (IL-2), IL-4, IL-17, interferon-γ (IFNγ) and tumour necrosis factor (TNF) [8]. The electrical driving force for Ca²⁺ entry through CRAC channels is determined by the resting potential of T cells, which is maintained predominantly by Kv1.3 and
KCa3.1 K⁺ channels. The activation of these channels provide the counterbalancing K⁺ efflux required for the maintenance of a negative membrane potential and that of the Ca²⁺ influx.

Kv1.3, KCa3.1 and CRAC channels are integral components of the IS signalosome [7,10,21–23]. These channels promote outside–in signalling, provide localized and polarized regulation of membrane potential and calcium signalling [24,25], modulate K⁺ dependence of integrin function [26,27], and serve to tether the channels to the T-cell receptor complex and intracellular signalling molecules via interactions with hDlg/SAP-97, p56lk tyrosine kinase [28], ZIP1 [10] and PSD-95 [29].

(iv) Target cell lysis by cytotoxic lymphocytes critically depends on the function of Ca²⁺-release-activated Ca²⁺ channels

Target cell lysis by cytotoxic lymphocytes is initiated by the recognition of the tumour antigens presented to CD8⁺ cytotoxic T cells (CTLs) in an MHC-I restricted manner. This leads to the destruction of the target cells in the kiss of death synopsis [30], whereby perforin and granzyme from cytolytic granules are released into the target cells, including tumour cells. Changes in the cytosolic Ca²⁺ concentration, especially locally at the immune synapse, are critically important for the complex operation of CTLs [15]. Compelling evidence shows that the Ca²⁺ influx that follows the interaction of CTLs with target cells and the release of Ca²⁺ from the ER store is mediated by the CRAC channel [31]. Functional CRAC channels and the consequent increase in the cytosolic Ca²⁺ may be needed in order to home to sites of inflammation or tumour development. For example, the adhesion molecule LFA-1 is required for adhesion of CTLs to target cells [32] and its activation is dependent on elevated levels of cytoplasmic Ca²⁺ [33], and vice versa, activated LFA-1 may regulate CRAC function by mitochondria recruited into the IS [34]. The key event in CTL function is, however, the transport of lytic vesicles to the IS and their fusion with the plasma membrane at the site of CTL/target cell interaction. Earlier studies indicated that elevated cytoplasmic levels of Ca²⁺ are required for the polarization of cytotoxic granules to the killing synapse during contact between CTLs and their targets (reviewed in [35]). However, a recent study by Maul-Pavicic et al. [36] clearly demonstrated that the cytotoxic granule polarization is not impaired by ORAI1 deficiency in human natural killer (NK) cells, whereas lytic granule exocytosis requires CRAC channels: degranulation is severely impaired in the absence of either ORAI1 or STIM1. Whether or not vesicle exocytosis is the only function depending on CRAC in CTLs, it was shown recently that functional CRAC is required for impeding engraftment of tumour cells and tumour growth in mice ([37], see also below).

(b) Ion channels in the innate immunity

The physiological role of ion channels ion is also substantial in the innate immunity. Monocytes and microglia (resident macrophages in the central nervous system) express voltage-gated Kv1.3 and Ca²⁺-activated KCa3.1 K⁺ channels, similar to T lymphocytes. In addition, Kv1.5 is also expressed in variable extent in both macrophages and microglia [38,39], and Kv1.3/Kv1.5 heterotetrameric channels are important determinants of the plasma membrane K⁺ conductance in macrophages [40]. The K⁺ channels, similar to T cells, regulate the membrane potential of macrophages and microglia, and thus the Ca²⁺ influx pathway through CRAC channels. Pharmacological interference with the channels, knockdown of the expression of the K⁺ channels or use of KO animals led to the conclusion that the inhibition of the channel function leads to impaired activation and cytokine secretion of these cells. The ion channel repertoire of microglia is extended by the expression of tetrodotoxin (TTX)-sensitive voltage-gated Nav1.6 Na⁺ channels, where the inhibition of these channels with TTX significantly reduced the phagocytic, which led to the improvement of neuroinflammatory diseases in mice [41]. Similarly, the expression of voltage-gated Nav1.7 Na⁺ channels was reported [42] and characterized in human monocyte-derived immature dendritic cells. The presence of Nav1.7 seems to depolarize the membrane potential of immature dendritic cells and stimulate cell migration, whereas pharmacological inhibition of Nav1.7 by TTX sensitized the immature dendritic cells for activation signals [43]. NK lymphocytes that play important roles in the control of tumours and viral infections express K⁺ channels identified as Kv1.3 and KCa3.1 [44,45]. Interestingly, as found with T and B lymphocytes (see below), the relative numbers of Kv1.3 and KCa3.1 vary between subpopulations of NK cells and confer different sensitivities to selective blockers of these channels [45]. Indeed, following incubation with the cytokines IL-2 and IL-15, plastic-adherent NK cells upregulate KCa3.1 channels, whereas non-adherent NK cells upregulate Kv1.3 channels. The critical role of the CRAC channel in controlling lytic granule exocytosis in NK cells was mentioned earlier (see above). Kv and KcA channels were also identified in neutrophils [46].

3. Subset-specific expression of ion channels in lymphocytes

Although the ion channel repertoire of various T and B lymphocyte subpopulations have been characterized [47,48], the functional consequence of the subset-specific expression of ion channels in B cells is less obvious regarding anti-cancer immunity, thus, we restrict our description to T cells.

(a) Subsets of T lymphocytes

Fully mature human T lymphocytes can be separated into three subpopulations based on their surface expression of the phosphatase CD45RA and the chemokine receptor CCR7 [49]. Naïve T cells express both markers and are CD45RA⁺CCR7⁺. Long-lived central memory T (T CM) lymphocytes, the largest pool of circulating memory T lymphocytes, maintain the expression of CCR7 but lose the expression of CD45RA and are therefore CD45RA⁻CCR7⁺. The third population of T cells is composed of effector memory T (TEM) lymphocytes, which represent fewer than 20% of circulating T cells and are negative for the expression of either marker (CD45RA⁻CCR7⁻).

Another subset of T lymphocytes consists of regulatory T cells (T reg) that play important roles in anti-tumour immunity by reducing inflammation. In humans, T reg cells can be identified by the following cell-surface phenotype: CD3⁺ CD4⁺ CD25highCD127low.

(b) Subset-specific expression of K⁺ channels in T lymphocytes

Quiescent naïve, TEM, TEM, and T reg lymphocytes express 200–300 Kv1.3 and 5–35 KCa3.1 channels per cell [47,50]. However, activation with either an antigen or a mitogen
induces drastic changes in K⁺ channel expression in the T lymphocyte subpopulations, leading to a different K⁺ channel phenotype. CCR7⁺ naive and TEM cells upregulate KCa3.1 channels to approximately 500/cell with little change in Kv1.3 channel expression [47,51]. By contrast, CCR7⁺ TEM cells increase Kv1.3 expression to 1500 channels/cell with little to no change in KCa3.1 levels. This differential upregulation of one K⁺ channel or the other was identified in both CD4⁺ and CD8⁺ T cells [47]. This switch in K⁺ channel expression significantly affects responsiveness to selective blockers of Kv1.3 and KCa3.1 channels as Kv1.3 channel blockers preferentially inhibit CCR7⁻ TEM lymphocytes, whereas KCa3.1 channel blockers mainly target CCR7⁺ naive/TEM lymphocytes [52–54]. The subset specific to ion channels allows a differential inhibition of the function of T-cell subsets, which has therapeutic advantage in autoimmune diseases as well as in anti-tumour immunity, as discussed below.

4. Blockers of ion channels in immune cells

Ion channel blockers are valuable pharmacological tools to dissect the physiological role of an ion channel or interfere with the pathophysiology of a disease, which requires the development of high affinity and specificity blockers. Despite the efforts, the development of CRAC blockers lags behind that of K⁺ channel blockers regarding both the affinity and specificity, and thus we discuss here briefly the K⁺ channel blockers only.

(a) Blockers of the voltage-gated K⁺ channel Kv1.3

Molecules having high affinity and specificity for Kv1.3 belong to two major groups: small organic molecules and peptide blockers of the channels.

K⁺ channel-blocking peptide toxins consists of 20–35 amino acids, their rigid structure is stabilized by disulfide bridges, and inhibit the K⁺ channels by a simple pore-blocking mechanism similar to a cork in the bottle [9]. Very potent natural toxins with picomolar Kv1.3 affinity were isolated from scorpions, such as Vm24 (α-KTx 23.1) [55,56] and OSK1 [57], and from the sea anemone Stichodactyla helianthus, ShK [58,59]. Vm24 has an exceptional pharmacological profile as being more than 100-fold selective for Kv1.3 over other voltage- and Ca²⁺-activated K⁺ channels tested [55], whereas other peptides had to be modified in order to increase selectivity for Kv1.3. The most successful mutant peptides of ShK, ShK-Dap22, ShK-F6CA and ShK-186 [53], successfully suppress TEM-dependent autoimmune diseases by interfering with T-cell activation, Ca²⁺ signalling, production of IL-2 and IFNγ, and efficiently inhibiting the proliferation of CCR7⁻ TEM lymphocytes and the activation of integrin β1, which plays an important role in the motility of activated TEM [10,60]. Similar biological effects of Vm24 were also reported, thus putting these molecules into the front line of specific interference with the function of a T-cell subset. The potential role of the toxin-like domain (TxD) in the matrix metalloproteinase (MMP) MMP23 (MMP23-TxD), which is evolutionarily related to ShK, in suppressing anti-cancer immunity is discussed later in the review.

Non-peptide, small-molecule inhibitors of Kv1.3 were identified with very diverse chemical structures [9]. Some of these molecules were also developed on a natural template, e.g. the K⁺ channel blocking component of Ruta graveolens alkaloids, 5-methoxypsoralen was chemically modified to generate a series of Kv1.3 inhibitors including Psora-4 [61] and PAP-1 [62] with nanomolar affinity for Kv1.3 and good selectivity over other K⁺ channels. The therapeutic applications of PAP-1 for inhibiting specific immune reactions have also been shown in animal models of skin diseases [63].

(b) Blockers of the Ca²⁺-activated K⁺ channel KCa3.1

Similar to Kv1.3 inhibitors, blockers of the intermediate conductance Ca²⁺-activated K⁺ channel, KCa3.1, can also be of peptide and non-peptide/small molecule origin [64,65]. Peptide toxins isolated from scorpions, such as charybdotoxin (ChTx) and maurotoxin (MTX), or their genetically engineered derivatives, e.g. ChTX-Glu32 [66], are high affinity inhibitors of KCa3.1 (approx. nM), although the lack the selectivity for KCa3.1 over other physiologically important channels. On the contrary, the development of small-molecule inhibitors of KCa3.1 turned out to be more successful resulting in high affinity and selectivity blockers. A significant fraction of these molecules were synthesized based on the clotrimazole template. TRAM-34, developed by Wulff et al. [67] inhibits KCa3.1 with an IC₅₀ of 20 nM and exhibits 200–500-fold selectivity over Kv channels and 1000-fold selectivity over KCa1.1 and KCa2.x channels without significant side effects. The 4-phenyl-1H-pyran-3,4-dione cyclohexadienes constitute another group of the nanomolar-affinity KCa3.1 inhibitors (as low as 1–10 nM IC₅₀), some of these compounds also show high selectivity for KCa3.1, for example cyclohexadienes lactone (reviewed in [65]). The increased cytotoxicity and tumour cell lysis by human NK cells upon KCa3.1 inhibition is discussed later in the review.

5. Involvement of ion channels in cancer cell – immune cell interactions: potential therapeutic applications

Although our knowledge about the expression of ion channels in various cells of the immune system is remarkable, most of our knowledge is related to the function of ion channels and their therapeutic targeting in acute or chronic inflammation and in autoimmune diseases. Relatively little is known about anti-tumour-immunity-specific functions of ion channels or targeting of these ion channels to fight the disease. In the subsequent paragraphs, we aim at summarizing the marginal literature in this field and based on the principles taken from the literature related to inflammation and ion channels we make an attempt to isolate ion channel targets for potential pharmacological interference.

Improving anti-cancer immunity, in principle, may mean an increase in the efficiency in the antigen presentation, recognition and tumour cell killing by the immune system or in the inhibition of the immunosuppressive tumour network existing in the TM. Both of these fundamental processes can be dramatically influenced by the function of the ion channels.

(a) Ca²⁺-release-activated Ca²⁺, Kv1.3 and KCa3.1 channels are required for tumour cell lysis by CD8⁺ cytotoxic T cells

CTLs play an important role in anti-tumour immune responses because of their ability to kill tumour cells [15].
Using mice with T-cell-specific deletion of Stim1 and Stim2 genes that lack CRAC channel function in CD4⁺ and CD8⁺ T cells, Feske and co-workers [37] showed that SOCE through CRAC was not required for the expansion of tumour-specific CTLs and their infiltration of tumours, but was necessary for lytic granule exocytosis, tumour cell killing and the production of IFNγ and TNFα. Thus, functional CRAC channels seem to have beneficial effects on CTL functions, and thus anti-tumour immunity.

T lymphocytes play important roles in the detection and destruction of tumour cells. Solid tumour environments are hypoxic, and hypoxia was shown to inhibit the function of T lymphocytes. This inhibition is associated with the down-regulation of the surface expression of Kᵥ1.3 channels and retention of the channels in the trans-Golgi by T lymphocytes under hypoxic conditions [68,69].

In patients with metastatic colorectal cancer, the detection of high numbers of infiltrating CD8⁺CCR7⁻ TEM lymphocytes represents a favourable prognosis factor, regardless of the number of infiltrating Treg cells [70]. By contrast, in an acute model of cancer mice deficient in CCR7, were unable to reject MHC class I mismatched tumour cells at least in part because of a reduced allogeneic response of the CD8⁺ T lymphocytes [71]. These results, taken together with our knowledge of the role of Kᵥ1.3 channels in CCR7⁺ and CCR7⁻ T lymphocytes, suggest that blocking Kᵥ1.3 channels may affect T-cell responses to metastatic cancer, whereas blocking KCa3.1 channels may affect T-cell responses to primary tumours.

(b) Lack of functional Ca²⁺-release-activated Ca²⁺ channels inhibits Treg development

Regulatory T cells, under physiological conditions, are crucial for maintaining T-cell tolerance to self-antigens, and thus protect the organism from autoimmune reactions. A dominant component of the immunosuppressive network in the TME is constitutive Treg, which suppress anti-tumour effector T cells by producing immunosuppressive cytokines TGF-β and interleukin 10 [72]. Treg depletion improves endogenous anti-tumour immunity and the efficacy of active immunotherapy in animal models for cancer, suggesting that inhibiting Treg function could also improve the limited successes of human cancer immunotherapy [73]. There are different strategies to block Treg activity: depletion, interference with trafficking, inhibition of differentiation, blockade of function or raising the effector T-cell threshold for suppression. Some of these depend on the activity of ion channels. Oh-Hora and colleagues showed that T-cell-specific ablation of both STIM1 and STIM2 resulted in a notable lymphoproliferative phenotype and a selective decrease in regulatory T-cell numbers. They concluded that the SOCE promoted by STIM proteins is required for the development and function of regulatory T cells. A recent study also showed that STIM-dependent SOCE speculatively regulates the thymic development of agonist-selected T cells, which includes regulatory T cells and invariant NK T cells [74], another component of the immunosuppressive cells reducing anti-cancer immunity [5].

It seems thus that CRAC channel function promotes proper CTL function and the development of Treg, thus, on one hand SOCE stimulates the efficiency of anti-cancer immunity, on the other hand inhibits it. In the absence of T-cell-subset-specific expression CRAC channels, the selective targeting of Treg versus CTLs is questionable. As mentioned above, the properties of the CRAC-mediated long-lasting Ca²⁺ signal is heavily influenced by the membrane potential of the cells [7,8]. If Kᵥ channels contributing to the maintenance of the membrane potential were different in CTLs versus Treg then the Ca²⁺ signal could be selectively influenced in these cells through the inhibition of the proper Kᵥ channel. Direct electrophysiological measurements on tumour infiltrating lymphocytes are missing, and thus the Kᵥ channel repertoire of the cells can only be speculated from studies on peripheral blood lymphocytes. Unfortunately, CD8⁺ CTLs and Treg cells show similar Kᵥ1.3 dominance (i.e. small number of KCa3.1 channels per cell), the only small difference was the slightly greater membrane surface area of Treg, and consequently lower channel density than that of naive cells [50].

(c) Myeloid-derived suppressor cells are induced in a TRPV1-dependent manner

Terminally differentiated myeloid cells—macrophages, dendritic cells and granulocytes—are essential for the normal function of both the innate and adaptive immune systems. However, chronic inflammation in the tumour tissue alters myeloid cells [75] and can convert them into potent immunosuppressive cells [76]. In addition to Treg and iNKT, these MDSC contribute significantly to the immunosuppressive network in the TME. MDSC continuously produce inflammatory cytokines and mediators (IL-1, IL-6, reactive oxygen species and nitric oxide) [77]. Although direct electrophysiological studies are missing regarding MDSCs existing in tumours, one can assume that the ion channel dependence of these MDSC can be similar to those involved in inflammations. The first study in this topic was published recently by Hegde et al. [78] who showed that the activation of TRPV1 receptors can trigger MDSCs, which in turn, can inhibit inflammation and hepatitis in mouse. Transient receptor potential vanilloid 1 (TRPV1) is an ion channel that is activated by noxious heat, capsaicin and other diverse stimuli, including cannabidiol, the natural non-psychoactive cannabinoid [79]. TRPV1 is a non-selective cation channel that prefers Ca²⁺ over Na⁺. Cannabidiol failed to induce MDSCs and suppress hepatitis in the livers of vanilloid receptor-deficient mice (TRPV1⁻/⁻) thereby suggesting that CBD primarily acted via this receptor to induce MDSCs. As TRPV1 antagonists exist, the beneficial effects of TRPV1-inhibition and the anticipated decrease in MDCS-dependent immunosuppression over TRPV1-dependant inhibition of tumour growth [80] and other pharmacological effects should be compared experimentally.

(d) Blocking KCa3.1 increase cytotoxicity and tumour cell lysis by human NK cells

Owing to their natural ability to recognize and lyse tumour cells using a variety of recognition receptors, NK cells have been examined clinically in several immunotherapeutic strategies for cancer. Several modulators of NK functions, such as IL-2, IL-15, have been also been described [81]. A recent study from the Beeton laboratory showed that NK cell functions can also be modulated through the activity of plasma membrane ion channels [45]. Blocking KCa3.1 channels in adherent NK cells with the selective blockers TRAM-34 and NS6180 increases the proliferation of these cells as well as their ability...
to release cytotoxic granules and to kill tumour cells in vitro. The in vivo administration of TRAM-34 in a xenograft tumour model enhances the ability of adherent human NK cells to reduce the growth of a tumour of human origin. By contrast, the blockade of Kv1.3 channels had little to no effects on adherent NK cells but induced an inhibition of the proliferation and degranulation of non-adherent NK cells. These findings suggest that targeting KCa3.1 channels on NK cells may prove beneficial in cancer immunotherapy and that Kv1.3 channel blockers will not prevent tumour killing by adherent NK cells.

(e) The toxin-like domain of MMP23 blocks Kv1.3 function by multiple mechanisms

Extracellular proteases of the MMP protease family degrade ECM proteins, cleave cell-surface receptors, release apoptotic ligands, and activate chemokines and cytokines [82]. Their catalytic activity enables MMPs to participate in tissue remodelling, cell proliferation, cell migration, differentiation, angiogenesis, apoptosis and the immune response, all of which are important for tumour progression and metastasis [83]. One particular member of this family, MMP23, contains a small TxD which lies immediately after the catalytic domain and is joined to the C-terminal IgCAM domain of the protein by a short Pro-rich linker [84]. The MMP23-TxD is evolutionarily related to peptide K+ channel inhibitor toxins from sea anemones BgK and ShK. The solution NMR structure of the synthetic MMP23-TxD shows structural similarity to BgK and ShK, moreover, the soluble MMP23-TxD blocks voltage-gated K+ channels in the nanomolar to low micromolar range (Kv1.6 > Kv1.3 > Kv1.1 = Kv3.2 > Kv1.4, in decreasing order of potency) [84].

As MMP23-TxD blocks Kv1.3 (IC50 = 2.7 μM) and MMP23 is expressed in a variety of malignancies (e.g. breast cancer [85]), this raises the possibility of modulating the anti-cancer immunity by this peptide. For example, colon cancer cells secreting processed active MMP23 enzyme containing the Kv1.3 channel-blocking TxD may block Kv1.3 channels of infiltrating anti-tumour T cells [86] and suppress them as a means of immune evasion.

In addition to blocking the pore of Kv1.3 by MMP23-TxD the MMP23 pro-domain, which anchors it to the membrane using a type-II transmembrane domain, interacts with Kv1.3 in the endoplasmic reticulum and traps it intracellularly [86]. This decreases the cell-surface expression of Kv1.3, which might contribute to the regulation of cell proliferation of the tumour cells. A single protein, thus, might influence tumour cells and anti-tumour immunity simultaneously by interacting with ion channels by multiple mechanisms.

6. Conclusion

All immune cells analysed to date were found to express ion channels, including K+ and Ca2+ channels. These channels play important roles in regulating cell development and function through the regulation of membrane potential, cell migration and adhesion, production of chemokines and cytokines, and cytotoxicity towards tumour target cells. Use of ion channel blockers inhibits the function of specific cell sub-populations but, thanks to significant redundancy in anti-cancer immunity, such blockers have not been found to increase the incidence of tumours in animal models. For example, tumour-reactive CD8+ T-cell populations with the phenotypic and functional attributes of TCM are superior to TCM-derived effector T cells for adoptive immunotherapies [87], and considering the different ion channel expression of these cells and their blocker sensitivity, skewing the distribution of T cells toward TCM-derived CTLs might have therapeutic potential. It is also to be determined whether the biologically active molecules in the TM [5,6] directly interact/block ion channels of the immune system and inhibit anti-tumour immunity. Further understanding of the roles of those channels in the formation of killer synapse, granule polarization, and directed degranulation in CTL and NK cells may lead to the discovery of novel targets for improving anti-tumour immunity.

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