Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Modulating ion channel function with antibodies and nanobodies

Catelijne Stortelers[1], Carolina Pinto-Espinoza[2], Diane Van Hoorick[1] and Friedrich Koch-Nolte[2]

Immune cells express various voltage-gated and ligand-gated ion channels that mediate the influx and efflux of charged ions across the plasma membrane, thereby controlling the membrane potential and mediating intracellular signal transduction pathways. These channels thus present potential targets for experimental modulation of immune responses and for therapeutic interventions in immune disease. Small molecule drugs and natural toxins acting on ion channels have illustrated the potential therapeutic benefit of targeting ion channels on immune cells. Unwanted side effects and immunogenicity have however hampered the application of these molecules. Owing to their high specificity, low immunogenicity and beneficial pharmacodynamics, antibodies targeting membrane and secretory proteins have emerged as potent therapeutics in oncology and inflammation. Nanobodies — single domain fragments derived from heavy chain antibodies naturally occurring in camelids — offer additional benefits versus antibodies, including protrusion into cryptic epitopes and easy formatting of multi-specific reagents. Here we review recent progress in the development and application of antibodies and Nanobodies targeting ion channels on immune cells.

Addresses
[1] Ablynx nv, Technologiepark 21, B-9052 Zwijnaarde, Belgium
[2] Institute of Immunology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany

Corresponding author: Koch-Nolte, Friedrich (nolte@uke.de)

Current Opinion in Immunology 2018, **52**:18–26

This review comes from a themed issue on **Special section on Ion channels and immune cells**

Edited by **Pablo Pelegrín** and **Florence Velge-Roussel**

https://doi.org/10.1016/j.coi.2018.02.003

Introduction

Immune cells express various voltage-gated and ligand-gated ion channels that mediate the influx and efflux of charged ions across the plasma membrane [1,2•]. Channels for monovalent ions including potassium (K^+), sodium (Na^+), protons (H^+), and chloride (Cl^-) regulate the membrane potential and the cell volume. Channels for divalent cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and zinc (Zn^{2+}) play important roles in signaling pathways. For example, stimulation of antigen receptors on immune cells results in a rapid and sustained increase of intracellular Ca^{2+}. Genetic deficiencies in ion channels, so called channelopathies, are often associated with immune diseases. For example, mutations in the pore forming subunit of the Ca^{2+} release activated calcium channel ORAI1 are associated with immunodeficiency, life-threatening infections, and autoimmunity [3].

Small molecules targeting ion channels are established drugs in cardiovascular and neurologic diseases [4,5]. The L-type Ca^{2+} channel blocker nifedipine is a well known antihypertensive, the Na^+ channel blockers lidocaine and lamotrigine are used as a local anesthetic and anticonvulsant, respectively. Ivacaftor, a potentiator of the cystic fibrosis transmembrane conductance regulator responsible for Cl^- transport, shows benefit in cystic fibrosis. Ion channel inhibitors have revealed important roles of ionic signals also in innate and adaptive immune responses. Pharmacological inhibition of ion channels thus represent a potential novel pathway for immunomodulatory therapy [6]. A major drawback of small molecule drugs is their imperfect specificity, that is, they often target multiple members of a protein family. In case of ion channels, this can lead to serious cardiologic and neurologic side effects and result in dose-limited suboptimal efficacy.

Due to their extraordinary specificity, biologics offer an attractive strategy for overcoming these problems with the prospect to discriminate even closely related ion channel family members.

Nature has evolved potent biologics, that is, proteins and peptides, targeting ion channels. Well studied examples include peptidergic toxins produced by snails, scorpions and spiders [7,8•]. Despite reported issues with manufacturability and stability, several toxin derived peptides have advanced towards the clinic. For example, recently completed clinical studies with ShK-168 (Dalazatide), an K^+ channel blocking sea anemone toxin variant, have shown lasting improvement of psoriasis lesions with an acceptable toxicity and immunogenicity profile [9]. Ziconotide, a 25-amino acid Ca^{2+}-channel blocking peptide derived from a snail toxin, is in the clinic for treatment of severe pain in terminal cancer patients [10]. Ziconotide,

Box 1 Targeting ion channels on immune cells with Nanobodies

Venomous animal toxins provide striking examples for the capacity of proteins to modulate ion channel function. It is therefore not far fetched that the function of ion channels can similarly be modulated by antibody-based biologics. Akin to conventional antibodies, Nanobodies usually display exquisite selectivity for their target antigen. Nanobodies derived from immunized camelids display a particular propensity to bind to functional crevices on their target protein, and they may therefore outperform conventional antibodies as ion channel modulating agents, as illustrated in two recent proof of principle studies with the ligand gated P2X7 and the voltage gated Kv1.3 ion channels. As biologics, Nanobodies do not suffer from conversion to toxic metabolites, and, like antibodies, typically do not display any off-target side effects. Nanobodies usually are administered by intravenous or subcutaneous injection. For topical applications, Nanobodies can be delivered via inhalation to the respiratory tract, as cremes to the skin, or as capsules to the gastrointestinal system. Akin to antibodies, Nanobodies show little capacity to pass the blood–brain-barrier. This can be an advantage for avoiding unwanted on-target side effects in the CNS. If targeting of immune cells in the CNS is therapeutically desired, Nanobodies need further engineering for enhanced permeation of the blood–brain-barrier, for example, by increasing the isoelectric point or by genetic fusion to a ligand for a transcytosis receptor such as the transferrin receptor. Owing to their small size and excellent solubility Nanobodies can readily be linked genetically to other Nanobodies in order to adjust their circulation half life or to optimize the specificity for a particular cell population. The favourable biochemical properties of Nanobodies allow high concentration formulations and ensure favourable clinical developability. Nanobodies thus are excellent alternatives to venomous animal toxins as therapeutics for ion channel diseases and as immunomodulatory drugs.

however, must be administered intrathecally and is associated with severe side effects. A drawback small peptides generally have is a very short and unpredictable serum half-life. With their low immunogenicity, high selectivity, and favourable half-life antibodies and Nanobodies represent attractive alternatives to toxin-based biologics (Box 1) [11•]. Raising functionally active antibodies and Nanobodies against ion channels, however, remains notoriously challenging due to their conformationally dynamic nature and minimal surface area [12,13,14•].

In contrast to small molecule drugs, antibodies typically demonstrate exquisite specificity for a single protein target that rarely can be matched by small molecules (Box 1) [15]. Antibodies targeting membrane proteins, cytokines and growth factors are established therapeutics in oncology and inflammation [13,16,17]. With a size of 150 kilo Dalton (kD), however, conventional antibodies are rather large molecules when compared to the exposed surface of ion channels (Figure 1). Nanobodies retain the exquisite specificity of antibodies in a small and more flexible format [18].

Camelids have evolved antibodies composed only of heavy chains, in which the antigen binding domain is composed of just one variable domain, designated V_HH or Nanobody [18]. Nanobodies are therapeutic proteins based on single-domain antibody fragments. As compared to antibodies, V_HH paratopes have acquired a higher structural complexity by involving more residues in antigen binding compared to classical VHs to compensate for the loss of VL domains. The complementarity determining regions (CDR) of Nanobodies are typically longer, more protruding, and lack steric interference from the light chain. Hence Nanobodies can beamenable to targeting cryptic epitopes and crevices on proteins that are inaccessible to conventional antibodies. Nanobodies are easily cloned and expressed as recombinant proteins that are highly soluble, robust, and easily converted into multivalent and multispecific formats by genetic fusion using flexible linkers. Since ion channels are multimeric proteins, an optimized multivalent format can increase potency by avidity. The option for simultaneous binding to different epitopes in one molecule provides an additional advantage, especially for targets with high conformational flexibility such as ion channels and GPCRs. For example, potency and efficacy of a bi-specific CXCR2-specific Nanobody-dimer that targets both the linear N-terminus and extracellular loop 2 proved superior to simple bivalent monospecific formats [19].

Conventional antibodies targeting ion channels

Conventional antibodies that modulate ion channel function have been successfully generated in immunized rodents and rabbits against both, ligand-gated and voltage-gated ion channels [13,14•]. For example, monoclonal antibodies (mAbs) have been raised against the pro-inflammatory ATP-gated P2X7 ion channel [20,21] in rats immunized with mouse mast cells and in mice immunized with human P2X7-expressing cells. Both, the human P2X7-specific mAb L4 and the mouse P2X7-specific mAb 1F11 inhibited ATP-induced gating of P2X7. Therapeutic benefit was confirmed for systemically administrated mAb 1F11 in a mouse model of inflammatory colitis [21].

Voltage-gated and Ca^{2+}-release-gated ion channels are more difficult antibody targets than ligand-gated ion channels, as the former channels are formed by multiple membrane-spanning proteins with only tiny extracellular loops that cluster into a multimeric channel embedded into the plasma membrane. Several studies have reported the generation of human ion channel blocking antibodies, usually by immunization with peptides corresponding to an extracellular loop of the ion channel. For example, specific mouse mAbs were generated to human KCNK9 using its M1P1 loop fused to the Fc domain of IgG2a [22••], and to Orai1 using its second extracellular loop conjugated to bovine serum albumin [23•,24,25•]. Inhibition of ion channel function by KCNK9-specific mAb Y4 or by Orai1-specific mAb 10F8, however, required prolonged preincubation of cells expressing the respective ion channel with the antibody in order to induce internalization of the channel. In other words, both antibodies