TWIK-related Acid-sensitive K⁺ Channel 1 (TASK1) and TASK3 Critically Influence T Lymphocyte Effector Functions*

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Two major K⁺ channels are expressed in T cells, (i) the voltage-dependent Kᵥ1.3 channel and (ii) the Ca²⁺-activated K⁺ channel KCa 3.1 (IKCa channel). Both critically influence T cell effector functions in vitro and animal models in vivo. Here we identify and characterize TWIK-related acid-sensitive potassium channel 1 (TASK1) and TASK3 as an important third K⁺ conductance on T lymphocytes. T lymphocytes constitutively express TASK1 and -3 protein. Application of semi-selective TASK blockers resulted in a significant reduction of cytokine production and cell proliferation. Interference with TASK channels on CD3⁺ T cells revealed a dose-dependent reduction (~40%) of an outward current in patch clamp recordings indicative of TASK channels, a finding confirmed by computational modeling. In vivo relevance of our findings was addressed in an experimental model of multiple sclerosis, adaptive transfer experimental autoimmune encephalomyelitis. Pre-treatment of myelin basic protein-specific encephalitogenic T lymphocytes with TASK modulators was associated with significant amelioration of the disease course in Lewis rats. These data introduce K₉P channels as novel potassium conductance on T lymphocytes critically influencing T cell effector function and identify a possible molecular target for immunomodulation in T cell-mediated autoimmune disorders.

The last decade has revealed much knowledge about the intracellular events accompanied by T lymphocyte activation following recognition of antigens bound to major histocompatibility complexes. K⁺ selective ion channels in T cells and their role in immune responses have been discussed for decades, since the discovery that non-selective K⁺ blockers could inhibit T cell proliferation in vitro (1–3). The role of K⁺ channels in the activation of T cells is pivotal, because opening the channels hyperpolarizes the membrane potential, which in turn leads to suppression of T cell effector function, e.g. decreased cytokine release and suppression of proliferation (11, 12). Underlining this finding, in vivo blockade of Kᵥ1.3 has been shown to mediate beneficial effects in experimental autoimmune encephalomyelitis (EAE), a rodent model for multiple sclerosis (12, 13). The importance of K⁺ channels has been further validated by research on human T lymphocytes, which revealed a differential expression patterns of Kᵥ1.3 and Kᵥ3.1 on specific T cell subtypes (14). Interestingly, “chronically activated” T cells induced by repeated antigenic challenges of the cells selectively and stably up-regulate Kᵥ1.3 channels (15). Furthermore, these cells could be defined as CCR₇⁻ CD45RA⁻ effector memory T lymphocytes (16), which are implicated in different autoimmune disorders (15). In agreement with that Kᵥ1.3 has been shown to be highly expressed in human myelin-reactive T cells of patients with multiple sclerosis (17) as well as in autoreactive T cells from patients with other autoimmune diseases such as type-1 diabetes or rheumatoid arthritis (18). Thus K⁺ channels, especially Kᵥ1.3, have emerged as attractive targets for highly selective immunosuppressive strategies applicable for example in the therapy of T cell-mediated autoimmune disorders (19).

Two-pore-domain K⁺ channels (Kᵥ,P, KCNK), often described as “background” or “leak” channels, play a pivotal role in ance calcium-activated potassium channel; FACS, fluorescence-activated cell sorter; CFSE, carboxyfluorescein diacetate succinimidyl ester; EAE, experimental autoimmune encephalomyelitis; DAPI, 4',6-diamidino-2-phenylindole; IFN, interferon; IL, interleukin; PBS, phosphate-buffered saline; MBP, myelin basic protein.

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* This work was supported by Interdisciplinary Clinical Research Center (IZKF) Wuerzburg N39-1 (to S. G. M. and H. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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³ Submitted in partial fulfillment of a doctoral thesis, Department of Neurology, University of Wuerzburg, Wuerzburg, Germany.

§ The abbreviations used are: CRAC, calcium release-activated calcium channel; Kᵥ1.3, voltage-gated potassium channel; IKCa1, intermediate conductance calcium-activated potassium channel; FACS, fluorescence-activated cell sorter; CFSE, carboxyfluorescein diacetate succinimidyl ester; EAE, experimental autoimmune encephalomyelitis; DAPI, 4',6-diamidino-2-phenylindole; IFN, interferon; IL, interleukin; PBS, phosphate-buffered saline; MBP, myelin basic protein.

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**TASK on T cells**

setting the resting membrane potential and modulating neuronal excitability (20–23). K$_p$ channels consist of four transmembrane domains arranged in tandem building two pores. Importantly, their action is mostly time and voltage independent (21). TASK1 and TASK3 channels, two functional members of the K$_p$ channel family, can be regulated by a diversity of stimuli (extracellular acidification, G$_q$ proteins, and muscarine (20, 22–24)), and exhibit insensitivity to “classical” potassium channel blockers (e.g. tetraethylammonium, 4-aminopyridine). Based on their specific electrophysiological properties, pharmacological profile, and functional impact on the resting membrane potential they might represent a relevant ionic conductance influencing basic T cell function. We therefore questioned whether K$_p$ channels are expressed on T lymphocytes and dissect their functional role on the level of electrophysiological properties and T cell effector function, both in vitro and in an animal model of multiple sclerosis in vivo.

**EXPERIMENTAL PROCEDURES**

**Materials and Reagents**—Charybdotoxin, bupivacaine, spermine, and ruthenium red (diluted in H$_2$O; Sigma), Psora-4 (DMSO; Carl Roth GmbH, Germany), and anandamide (EtOH; Tocris, Germany) were frozen as aliquots for further use. CD3/CD28 dynabeads for cell stimulation were obtained from Dynal Biotech (Karlsruhe, Germany). Annexin-FLUOS (Roche), propidium iodide (Calbiochem, Darmstadt, Germany), DAPI (Mercck, Darmstadt, Germany), and carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Invitrogen) were used for cell labeling.

**Isolation and Culture of Human T Cells**—Human T cells were purified from peripheral blood samples of healthy donors ($n = 49$). Peripheral blood mononuclear cells were prepared by density centrifugation (Lymphoprep, Axis-Shield, Oslo, Norway) according to the manufacturer’s instructions. This was followed by magnetic cell sorting (MACS®, CD3 Microbeads, Miltenyi Biotec, Karlsruhe, Germany) for CD3. Purity of CD3$^+$ T lymphocytes was $>98\%$ as assessed by flow cytometry. Cells were maintained in RPMI 1640 containing 10% human AB-serum, 25 mM HEPES, 1% glutamine, and 1% antibiotics.

**Assessment of T Cell Function, Pharmacological Blockades**—4 × 10$^6$ freshly isolated CD3$^+$ T lymphocytes were seeded in 1 ml of T cell medium per well. CD3/CD28 dynabeads (Dynal Biotech) were added at a T cell to bead ratio of 1:1. Channel blockers in different concentrations were applied in parallel to bead stimulation (K$_s$L3 blockers, 10 and 100 nM charybdotoxin, and 1, 10, and 100 nM Psora-4; TASK1/3 blocker, 30, 100, and 250 µM bupivacaine; TASK1 inhibitor, 3, 30, and 100 µM anandamide; TASK3 blockers, 50, 500 µM, and 1 mM spermine, and 100 nM, 1 µM, 10 µM ruthenium red). The solvent solution in the final experimental solution did not exceed 1%. Application of the solvent alone (1%) did not influence the analyzed parameters. Stimulation were all done in duplicates. After 24 h incubation at 37°C and 5% CO$_2$, cells were centrifuged and subjected to further analysis by flow cytometry (stainings for annexin V and propidium iodide). In parallel, supernatants were assessed for IFN$\gamma$ or IL2 protein levels by enzyme-linked immunosorbent assay (R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions.

**Flow Cytometry for TASK Channel Expression on T Lymphocytes**—Flow cytometry acquisition was done by standard methods. For antibody staining cells were resuspended in FACS® buffer (PBS containing 0.1% bovine serum albumin and 0.1% NaN$_3$), directly labeled antibodies were added. For annexin V/propridium iodide assays, cells were resuspended in annexin binding buffer (10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, and 0.18 mM CaCl$_2$) and stained with annexin-FLUOS (Roche) and propidium iodide (Calbiochem). For evaluation of TASK1/3 antibody specificity the following antibodies were used: rabbit anti-TASK1 (number P0981, Sigma), goat anti-TASK1 (number SC-32067), rabbit anti-TASK3 (number AB5721, Chemicon, Ochsenhausen, Germany), and goat anti-TASK3 (number SC-11320, Santa Cruz, Santa Cruz, CA) followed by the appropriate secondary antibodies (goat anti-rabbit fluorescein isothiocyanate or donkey anti-goat Cy3; Dianova, Hamburg, Germany) using standard protocols for extracellular (see above) and intracellular staining (permeabilization buffer and fixation buffer from ebioscience, San Diego, CA). Data acquisition was done using a FACS Calibur system (BD Biosciences). Results were analyzed using CellQuest Pro Software (BD Biosciences).

**T Cell Proliferation Assay in the Presence and Absence of TASK Channel Modulators**—Human T cells were labeled with 4 µM CFSE (Molecular Probes) for 10 min in the dark. RPMI containing 15% fetal calf serum was added for 20 min to stop labeling and was followed by three washing steps with RPMI, 10% fetal calf serum. CFSE-labeled cells were suspended in T cell medium and 1 × 10$^5$ cells per well were cultured at 37°C and 5% CO$_2$ in the presence of CD3/CD28 beads and ion channel modulators as described above. After 3 days of proliferation CFSE-labeled cells were washed and resuspended in FACS buffer. Proliferation assays were performed in duplicate and analyzed by flow cytometry calculating the responder frequency (dividing the number of dividing cells by total number of cells).

**Immunocytochemistry of Human and Rat T Cells**—Immunocytochemical stainings were performed on human T cells and rat myelin basic protein (MBP)-specific T cells. Cells were placed on coverslips coated with poly-l-lysine (Sigma) and fixed with 4% paraformaldehyde. Subsequently, cells were blocked with PBS containing 10% horse serum (PAA Laboratories, Cölbe, Germany), 2% bovine serum albumin, and 0.3% Triton X-100 overnight. Next, the primary antibodies (rabbit anti-TASK1, Sigma; rabbit anti-TASK3, Chemicon; rabbit anti-K$_s$L3, Chemicon) were added and incubated for 1 h. Cells were washed with PBS containing 0.3% Triton X-100 and incubated with secondary antibodies (Cy3-conjugated rabbit anti-goat, 1:100, Dianova) for another hour. Counterstaining of cell nuclei was performed using DAPI (0.5 µg/ml, Merck). Pictures were collected by immunofluorescence microscopy (Axioskop, Zeiss, Jena, Germany). Negative controls without the primary antibody revealed no positive signals (data not shown).

**Western Blot of T Cell Lysates**—Whole cell lysates from isolated purified T cells were used for Western blot analysis. In brief, cells were washed with ice-cold PBS, resuspended in lysis buffer (PBS containing 1% Triton X-100 and protease inhibitor mixture (Roche Diagnostics, Mannheim, Germany), and solubilized by sonification on ice. Cell lysates were centrifuged and
protein content in the clarified supernatant was measured by Bradford reaction. Samples (50 μg/lane for TASK1 and 100 μg/lane for TASK3) were subjected to 10% SDS-PAGE, followed by transfer to nitrocellulose membranes. Whole mouse brain (C57Bl6) was used as a positive control for TASK channel expression (23). Protein transfer was visualized by Ponceau S staining and membranes were then blocked with PBS containing 0.05% Tween 20 and 5% dry milk. The membranes were probed with rabbit anti-TASK1 (raised against the C-terminal part of the channel, 1:200; Sigma) or rabbit anti-TASK3 (polyclonal antibody against the C-terminal part of TASK3, 1:200; Chemicon), respectively. The secondary antibody was horse-radish peroxidase-conjugated donkey anti-rabbit (1:3,000; Amersham Biosciences). The antibody reaction was detected by enhanced chemiluminescence reaction (ECL, Amersham Biosciences).

**Electrophysiological Recording of Whole Cell Outward Currents in T Cells**—All experiments were conducted in whole cell configuration of the patch clamp technique. Individual human T lymphocytes were visually identified by infrared differential interference contrast video microscopy (25). Starting from a holding potential of −80 mV, membrane currents were recorded with pipettes pulled from borosilicate glass (GC150TF-10, Clark Electromedical Instruments, Kent, UK), connected to an EPC-10 amplifier (HEKA Elektronik, Langenbrecht, Germany), and filled with (in mM): K-gluconate, 95; K3-citrate, 20; NaCl, 10; HEPES, 10; MgCl2, 1; CaCl2, 0.5; 1,2-bis-(2-aminoxyethoxy)ethane-N,N,N',N'-tetraacetic acid, 1; Mg-ATP, 3; Na-GTP, 0.5. The internal solution was set to a pH of 7.25 with KOH and an osmolality of 295 mOsm/kg. Extracellular solution (in mM): NaCl, 120; KCl, 2.5; NaH2PO4, 1.25; HEPES, 30; MgSO4, 2; CaCl2, 2; dextrose, 10; pH 7.2, was adjusted with NaOH and osmolality was set to 305 mOsm/kg. Outward currents were elicited by repeated 500-ms pulses from −80 to 40 mV, applied at 30-s intervals. Typical electrode resistance was 3–6 meghms with an access resistance of 6–15 meghms. Series resistance compensation of more than 40% was routinely used. Voltage clamp experiments were governed by Pulse software (HEKA Elektronik) operating on an IBM compatible PC. A liquid junction potential of 6 ± 2 mV (n = 6) was measured and taken into account according to Neher (26).

**Adoptive Transfer Experimental Autoimmune Encephalomyelitis**—Female Lewis rats (150–160 g) were kept at standard conditions with free access to food and water. All animal experiments were approved by local authorities and conducted according to the German law of animal protection. Experiments were approved by local authorities and conducted according to the German law of animal protection. The calcium-dependent potassium current IC originates from the membrane mechanism (23). Starting from a holding potential of 0 mV, membrane currents were governed by Pulse software (HEKA Elektronik) operating on an IBM compatible PC. A liquid junction potential of 6 ± 2 mV (n = 6) was measured and taken into account according to Neher (26).

**Statistical Analysis**—All results are presented as mean ± S.E. Statistical analysis was performed using the Student’s t test modified for small samples as described previously (36). Statistical significance was set at p < 0.05, which is indicated by double asterisks.

**RESULTS**

**TASK1 and TASK3 Channels Are Expressed on Human T Lymphocytes**—In a first set of experiments purified human CD3+ T lymphocytes (n = 6 donors) were assessed by immu-
Expression of K^+ channels Kv1.3, TASK1, and TASK3 on human T lymphocytes. A, immunocytochemical staining of human CD3^+ T lymphocytes with DAPI (left panel), anti-Kv1.3 (middle panel), and overlay (right panel). B and C, antibodies raised against TASK1 (B, middle panel) and TASK3 (C, middle panel) and corresponding DAPI counterstaining (B and C, left panels) indicates a channel expression on human T lymphocytes (B and C, right panels). Scale bars represents 50 μm (upper left) and counts for all images (enlarged details scale bar, 10 μm). D, Western blot analysis using a TASK1-specific antibody revealed two distinct signals at 50 and 65 kDa (→). Left lane indicates marker bands. Lanes 1, negative control; 2, albumin blotting (no band); 3, whole mouse brain; and 4–6, lysates of CD3^+ positive isolated T cells. E, Western blot of TASK3 protein indicates a clear signal at 60 kDa (→) using CD3^+ positive isolated T cells of healthy humans (lanes 3–5). Marker bands are indicated on the left lane. Lanes 1, negative control; 2, whole mouse brain; and 3–5, three lysates of CD3^+ positive isolated T cells from 3 independent healthy donors. F, flow cytometry for TASK1 (upper panel) and TASK3 (lower panel). Fluorescence intensity is plotted against event counts. Dotted lines, unstained controls; thin black lines, staining with secondary antibodies alone; bold black lines, staining with TASK1/3 antibodies. Arrows (↑) indicate overlapping signals for thin and bold black lines. One of three representative experiments is shown.
FIGURE 2. Production of interferon γ and IL2 by human T lymphocytes is reduced in the presence of ion channel modulators against K⁺ channels Kv1.3, TASK1, and TASK3. A, cell viability assessment in the presence and absence of K⁺ channel modulators was performed by flow cytometry. Gating procedures (side and forward scatter), results under control conditions, and after treatment with H₂O₂ (100 μM) are depicted. B, upper panel, superimposed images exemplify flow cytometry analysis for apoptosis and necrosis under control conditions (con) and in the presence of the different channel modulators charybdotoxin (100 nM, C) and Psora-4 (100 nM, P). Lower panel, interferon γ production of isolated human T lymphocytes after stimulation with CD3/CD28 beads under control conditions (positive control; black column, +) and in the presence of the Kv1.3 channel inhibitors charybdotoxin (C1, 10 nM; C2, 100 nM) and Psora-4 (P1, 1 nM; P2, 10 nM; P3, 100 nM). C, IFNγ production after application of bupivacaine (B1, 30 μM; B2, 100 μM; B3, 250 μM) and anandamide (A1, 3 μM; A2, 30 μM; A3, 100 μM). D, TASK3 specific inhibition by spermine (S1, 50 μM; S2, 500 μM; S3, 1 mM) and ruthenium red (R1, 100 nM; R2, 1 μM; R3, 10 μM). E, blockade of Kv1.3 and TASK channels using selected concentrations leads to a similar effect on IL-2 production. **, p < 0.05.

cols (Fig. 1F, left side). The same specificity was found for an anti-TASK1 antibody (intracellular target) by Santa Cruz, whereas anti-TASK3 (extracellular; Chemicon) revealed positive signals in intracellular and extracellular staining protocols (data not shown). Taken together, these results point to expression of K₂P channels TASK1 and -3 on human CD3⁺ T lymphocytes.

TASK1 and TASK3 Modulate T Cell Effector Function: Pharmacological Blockade Inhibits IFNγ Secretion, IL2 Secretion, and T Cell Proliferation—To correlate K₂P channel expression to T cell effector functions we next studied the influence of pharmacological blocking reagents for K⁺ channels on cytokine production and T cell proliferation. Human CD3⁺ T cells were stimulated with CD3/CD28 beads. Levels of IFNγ and IL2 secreted in the supernatants were assessed in response to application of different ion channel modulators. To exclude unspecific effects of the compounds on cell viability, each inhibitor was titrated and tested by annexin V/propidium iodide staining to estimate T cell apoptosis and necrosis. The gating procedure, results under control conditions, and after treatment with H₂O₂ (100 μM, positive control for cell death) are depicted in Fig. 2A. Charybdotoxin (C, 10 nM, 100 nM) and Psora-4 (P, 1, 10, and 100 nM), both inhibitors of Kv1.3 channels, the TASK channel inhibitor bupivacaine (B, 30, 100, and 250 μM), and anandamide (A, 3, 30, and 100 μM) as well as the TASK3 modulating compounds spermine (S, 50 μM, 500 μM, and 1 mM) and ruthenium red (R, 100 nM, 1 μM, and 10 μM) had no significant direct effect on cell survival (Fig. 2, B–D, upper panel) as compared with control conditions (con). Charybdotoxin and Psora-4, both modulators of Kv1.3 were used as positive controls (38). Application of 100 nM charybdotoxin significantly reduced IFNγ production to 56 ± 3% (n = 5, p = 0.0002; 10 nM: no effect, n = 5, p = 0.76; Fig. 2B; C1, C2). Kv1.3 channel modulation by Psora-4 significantly lowered IFNγ amounts in the supernatant at all concentrations used (Fig. 2B and Table 1). TASK channel modulation using bupivacaine (23) significantly decreased IFNγ levels at concentrations ≥100 μM (Fig. 2C and Table 1). TASK channel inhibition by the
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**TABLE 1**

| Substance       | Abbreviation | Concentration | Result | n  | p value |
|-----------------|--------------|---------------|--------|----|---------|
| Psora-4         | P1           | 1 nM          | 64 ± 8% | 5  | 0.01    |
|                 | P2           | 10 nM         | 61 ± 8% | 5  | 0.007   |
|                 | P3           | 100 nM        | 54 ± 12%| 5  | 0.02    |
| Bupivacaine     | B1           | 30 µM         | 96 ± 4% | 5  | 0.44    |
|                 | B2           | 100 µM        | 74 ± 6% | 5  | 0.01    |
|                 | B3           | 250 µM        | 59 ± 12%| 5  | 0.00001 |
| Anandamide      | A1           | 3 µM          | 81 ± 4% | 5  | 0.01    |
|                 | A2           | 30 µM         | 68 ± 8% | 5  | 0.02    |
|                 | A3           | 100 µM        | 26 ± 2% | 5  | 0.00001 |
| Spermine        | S1           | 50 µM         | 82 ± 8% | 5  | 0.1     |
|                 | S2           | 500 µM        | 62 ± 4% | 5  | 0.001   |
|                 | S3           | 1 mM          | 55 ± 5% | 5  | 0.0007  |
| Ruthenium red   | R1           | 100 nM        | 47 ± 6% | 5  | 0.0007  |
|                 | R2           | 1 µM          | 40 ± 8% | 5  | 0.002   |
|                 | R3           | 10 µM         | 33 ± 6% | 5  | 0.0005  |

**FIGURE 3.** Modulation of TASK inhibits proliferation of human T lymphocytes. Human CD3 T lymphocytes were labeled with CFSE and cultured in the presence of CD3/CD28 beads (A) and various K⁺ channel modulators (B–D). Application of the potassium channel modulators Psora-4 (P, 10 nM, n = 5), anandamide (A, 30 µM, n = 5), and ruthenium red (R, 100 nM, n = 5) indicated a marked reduction of T cell proliferation. Exemplary histograms are shown. See corresponding Table 2.

| Substance       | Abbreviation | Concentration | Result | n  | p value |
|-----------------|--------------|---------------|--------|----|---------|
| Bupivacaine     | B1           | 30 µM         | 96 ± 4% | 5  | 0.44    |
|                 | B2           | 100 µM        | 74 ± 6% | 5  | 0.01    |
|                 | B3           | 250 µM        | 59 ± 12%| 5  | 0.00001 |
| Anandamide      | A1           | 3 µM          | 81 ± 4% | 5  | 0.01    |
|                 | A2           | 30 µM         | 68 ± 8% | 5  | 0.02    |
|                 | A3           | 100 µM        | 26 ± 2% | 5  | 0.00001 |
| Spermine        | S1           | 50 µM         | 82 ± 8% | 5  | 0.1     |
|                 | S2           | 500 µM        | 62 ± 4% | 5  | 0.001   |
|                 | S3           | 1 mM          | 55 ± 5% | 5  | 0.0007  |
| Ruthenium red   | R1           | 100 nM        | 47 ± 6% | 5  | 0.0007  |
|                 | R2           | 1 µM          | 40 ± 8% | 5  | 0.002   |
|                 | R3           | 10 µM         | 33 ± 6% | 5  | 0.0005  |

**TABLE 2**

The table summarizes all investigated compounds and concentrations of the inhibitors used in this study concerning human T-cell proliferation

| Substance       | Result | n  | p value |
|-----------------|--------|----|---------|
| Unstimulated    | 6      | 1  | 0.00001 |
| CD3/CD28 stimulated | 100   | 0  | 0       |
| Charybdotoxin, 100 nM | 61    | 6  | 0.002   |
| Psora-4, 10 nM  | 27     | 7  | 0.004   |
| Bupivacaine, 250 µM | 52    | 8  | 0.004   |
| Anandamide, 30 µM | 70    | 9  | 0.02    |
| Spermine, 500 µM | 67     | 12 | 0.046   |
| Spermine, 1 nM  | 61     | 11 | 0.02    |
| Ruthenium red, 1 nM | 98    | 4  | 0.46    |
| Ruthenium red, 10 nM | 76    | 4  | 0.003   |
| Ruthenium red, 0.1 µM | 28   | 8  | 0.0007  |

Psora-4) significantly reduced responder frequency after bead stimulation (n = 5 each; Fig. 3B, Table 2). TASK channel modulation by bupivacaine (n = 5; Table 2), anandamide (n = 5; Fig. 3C, Table 2), spermine (TASK3, n = 5; Table 2), and ruthenium red (TASK3, n = 5; Fig. 3D and Table 2) significantly suppressed T cell proliferation (30–70%; Table 2).

Electrophysiological Recordings Reveal the Contribution of TASK to the Potassium Outward Current in Human T Cells—In the next set of experiments whole cell patch clamp recordings of purified CD3⁺ human T lymphocytes were used to evaluate the contribution of TASK channels to the potassium outward current of T cells (n = 31 donors; multiple single cell recordings of each donor). Stepping the membrane potential from −80 to +40 mV (Fig. 4A, left panel, inset) under voltage-clamp conditions evoked an outward current of 424 pA (range: 213–944 pA). Application of the voltage protocol at 30-s intervals indicated a stable outward current over time and current rundown could by analyzed as 6 ± 2% (Fig. 4, A, left panel; C, left and middle panel, n = 6). Calculation of the underlying time constant (τ) under control conditions revealed a single exponential decay with 195 ± 15 ms (Fig. 4, A, left panel; C, right panel and inset; n = 6). However, addition of the well established Kᵥ1.3 channel inhibitor Psora-4 (100 nM) immediately reduced the outward current by 54.8 ± 8.8% (Fig. 4A, right panel, gray trace; n = 4, p = 0.003) although the time constant was not significantly altered (τ: 217 ± 17.3 ms). In contrast, applying the TASK-specific modulator anandamide resulted in a rapid reduction of the K⁺ outward current (30 µM, 42 ± 10%, n = 4, p = 0.01; 100 µM, 44 ± 8%, n = 4, p = 0.0017; Fig. 4, B and C, left and middle panels) and an accelerated time constant (30 µM, τ = 80 ± 17 ms; n = 4, p = 0.0002; 100 µM, τ = 69 ± 10 ms; n = 4, p = 0.00001; 4C, right panel). These results for the first time demonstrate a contribution of currents through Kᵥ1.3 channels to outward currents in human T cells. Next, blocker-sensitive currents were analyzed after graphical subtraction of currents in the presence and absence of channel modulators. Expectedly, the Psora-4-sensitive current component (the current after application of Psora-4 was subtracted from the control current, Fig. 4A, right panel, black trace minus gray trace) revealed a rapid current onset upon stimulation and a slow inactivation kinetic indicative of the delayed rectifying current Kᵥ1.3 (Fig. 4D, left panel). However, the anandamide-sensitive current component (the current after application of anandamide was subtracted from the control current, Fig. 4B, left panel,
FIGURE 4. Whole cell patch clamp recordings of potassium outward currents in human T cells reveal a pharmacological profile indicative of \( K_{\text{V}.1.3} \) and TASK channels. A, voltage step protocols (see inset) from \(-80 \) to \(+40 \) mV (500 ms, repeated every 30 s) were used to evoke stable potassium outward currents on human T lymphocytes (left panel). Application of Psora-4 (100 nM, inhibitor of \( K_{\text{V}.1.3} \) channels) induced a clear current reduction (right panel). B, the endogenous cannabinoid anandamide (30 and 100 \( \mu \)M) significantly reduced the current. C, bar graph representation of rundown effects under control conditions (con) compared with TASK-specific inhibition by anandamide (30 \( \mu \)M, left panel). Amplitude of the net outward current plotted against time under control conditions (black squares, middle panel). Period of anandamide treatment (ana, gray circles) is indicated by a horizontal line (middle panel). Bar graph representation of time constants under control conditions and after application of anandamide (right panel) as indicated by mathematical single-exponential decay fits (right panel, inset). D, blocker-sensitive current components obtained by subtraction of currents after application of an inhibitor from control values. ** represents \( p < 0.05 \).

FIGURE 5. Effects of charybdotoxin and ruthenium red on the potassium outward currents in human T cells. A, effects of charybdotoxin (50 nM) on the net outward current are shown in the left panel. Mean bar graph representation (inset) and amplitude versus time plot of charybdotoxin (right panel) are shown. B, ruthenium red (RR) applied on the T cell outward current induced a significant current reduction at 1 and 10 \( \mu \)M (left panel). Amplitude of the net outward current plotted against time in the presence of 1 \( \mu \)M ruthenium red as indicated by a horizontal line (middle panel). Bar graph representation of ruthenium red effects on the net outward current of human T lymphocytes compared with control values (right panel). **, \( p < 0.05 \).

black trace minus gray trace) displayed a fast current onset with nearly no inactivation over the stimulation protocol, a typical feature of voltage-independent TASK channels (Fig. 4D, right panel). These findings were further corroborated by the electrophysiological fingerprint of charybdotoxin (50 nM), a second blocker of \( K_{\text{V}.1.3} \) (and KCa3.1), on human T lymphocytes (64.5 ± 6.4% reduction, \( n = 4, p = 0.0002 \); Fig. 5A, inset and right panel). The charybdotoxin-resistant currents (Fig. 5A, left panel) strongly resemble the properties of pure TASK currents, which provides further support for the coexistence of voltage-dependent and leak potassium currents on T cells. TASK3 modulation by ruthenium red (1 and 10 \( \mu \)M) induced a significant current reduction of 40.9 ± 8.5 and 52.6 ± 4\%, respectively (\( n = 4, p = 0.04/0.001 \); Fig. 5B). Taken together this data therefore clearly indicate the functional relevance of TASK channels on outward currents in human T lymphocytes and demonstrate the functional coexpression of \( K_{\text{V}.1.3} \) and TASK channels on these cells.

A Computational Model to Dissect the Contribution of Three K Channels (\( K_{\text{V}.1.3}, \text{KCa, and TASK} \)) for K\(^+\) Outward Currents—in the next experimental step we used a numeric model of human T cells to analyze the current components contributing to the resting membrane potential in human T cells. The model included the voltage-dependent \( K_{\text{V}.1.3} \) (\( I_{\text{K}_{\text{V}.1.3}} \)) channel (Fig. 6A), whereas inactivation and activation parameters were calculated according to the literature (31, 39). Based on these settings the currents evoked by a depolarizing voltage protocol (500 ms, from \(-50 \) to \(+50 \) mV, decrement 10 mV; Fig. 6A, inset) showed slow inactivation kinetics and characteristics as described for the net outward current of human T cells (16, 33). Next, the model was extended by the inclusion of a TASK current (\( I_{\text{TASK}} \)) as described earlier (34). TASK current alone resulted in a nearly voltage-independent model response to depolarizing voltage steps (Fig. 6B). Finally, we supplemented the model by including a Ca\(^{2+}\)-dependent K\(^+\) current (\( I_{\text{C}} \), Fig. 6C) (35). Based on our pharmacological results we assumed the contribution of all three conductances to the net outward current in human T cells and calculated 40% \( I_{\text{K}_{\text{V}.1.3}} \), 40% \( I_{\text{TASK}} \), and 20% \( I_{\text{C}} \). The composed current evoked by a depolarizing voltage step to \(+40 \) mV displayed typical features (e.g. slow inactivation) as recorded for human T lymphocytes (Fig. 6D, left panel). Mimicking Psora-4 actions (inhibitor of \( K_{\text{V}.1.3} \) channels as demonstrated by electrophysiological recordings) in the model reduced the \( K_{\text{V}.1.3} \) component to 0% resulting in a nearly voltage-independent current response evoked by a depolarizing voltage step (Figs. 4A, right panel, gray trace, and 6D, middle panel).
**TASK on T cells**

![Graphs and images showing computational modeling of K+ channel contributions to the net outward current in human T cells.](https://example.com/graphs)

**FIGURE 6.** Computational modeling of K+ channel contributions to the net outward current in human T cells. A single compartment cell model calculating the effects of K,1.3, TASK1, and 3 channels. We modeled a T cell outward current consisting of IKv1.3 (A), I_TASK (B), and I_2 (C). A–C, left column indicates the currents evoked by depolarizing voltage steps (inset) if only a single current is included in the model. Right panel demonstrates the activation and inactivation curve of Kv1.3 (A, right column), the current-voltage relationship of TASK channels (B, right column), and the activation curve of I_2 (C, right column). Analysis of the net outward current (40% IKv1.3, 40% I_TASK, and 20% I_2) demonstrated characteristic features comparable with the recorded outward current of human T lymphocytes (D, left panel). Different K+ channel contributions to the net outward current were modeled and revealed results comparable with the electrophysiological recordings under Psora-4 (D, middle panel) and anandamide (D, right panel).

This finding further corroborates our electrophysiological data above, suggesting a marked contribution of TASK channels to the outward current of human T cells. In a last step we modeled anandamide actions (TASK channel inhibitor as demonstrated by electrophysiological recordings) by reducing I_TASK to 0%. The remaining current component represents Kv1.3 channels (Figs. 4B, left panel, gray trace, and 6D, right panel). Recapitulating these findings from a numerical cell model support the results from electrophysiological recordings showing the functional coexpression of TASK and Kv1.3 channels in human T lymphocytes.

**Selective Blockade of T Lymphocyte TASK Channels Ameliorates Experimental Autoimmune Encephalomyelitis, a Model of**

**Multiple Sclerosis**—Guided by our data on the functional impact of TASK on T cell activation in vitro and our patch clamp results, we challenged the question whether selective modulation of T lymphocyte TASK channels modulates T cell-mediated inflammatory disorders in vivo. We therefore chose adoptive transfer experimental autoimmune encephalomyelitis, a well established animal model of multiple sclerosis in Lewis rats. To evaluate the effect of TASK modulation on T cells, we incubated encephalitogenic MBP-specific T lymphocytes with the K+ channel modulators (10 nM Psora-4, 100 nM charybdotoxin, and 30 μM anandamide) prior to adoptive transfer in rats. Expression of K,1.3 as well as TASK1 could be demonstrated on MBP-specific rat T lymphocytes by immunocytochemistry, similar to our results observed in human T lymphocytes (Fig. 7A). Intravenous transfer of restimulated MBP-specific (6 × 10^6 cells/animal) T cells elicited a typical EAE with symptom onset at 2–3 days, disease maximum around day 5, and recovery from days 6–10 (onset, 3.3 ± 0.3 days, n = 8; disease maximum, score 4.7 ± 0.5, n = 8; decline to baseline, 10.4 ± 0.5 days, n = 8; Fig. 7, C–E). Incubation of encephalitogenic T cells with the Kv1.3 blocker Psora-4 24 h prior to transfer led to a slightly delayed onset of disease (not significant, ns, p = 0.09), amelioration of the disease maximum (score 3.0 ± 0.6, n = 8, p = 0.03), and faster recovery of symptoms (p = 0.03; Fig. 7C). Different results were seen when using charybdotoxin that showed no significant effect on the investigated parameters (onset, p = 0.25; disease maximum, score 4.8 ± 0.8, p = 0.74, n = 8; decline to baseline, p = 0.57; Fig. 7D). Selective blockade of TASK channels with anandamide ameliorated adoptive transfer experimental allergic encephalomyelitis. Although disease onset was delayed in the presence of anandamide (ns, p = 0.39), the maximum disease score was reduced significantly (2.8 ± 0.3, n = 9, p = 0.02). Furthermore, some animals recovered earlier (p = 0.03; Fig. 7E). In agreement with these results from EAE, MBP-specific T cells showed a significantly reduced IFNγ production in vitro when pretreated with Psora-4 (0.71 ± 0.08, p = 0.02, n = 5) and anandamide (0.66 ± 0.09, p = 0.01, n = 5), whereas charybdotoxin failed to have a lasting effect on
represent 10
Left column shows DAPI stainings, focused on 2 major K
autoimmune disorders (12, 16). Thus far, research reports have
vivo experiment). Pretreatment of encephalitogenic T cells MBP-TC with Psora-4 (C
voltage-dependent KV1.3 channel and (ii) the Ca2
-mediated disorder.
ological relevance of TASK channel modulation in a T cell-
obtained from the animal model demonstrate the pathophysi-
associated with obvious side effects. Taken together, results
in vivo was not
of all animals including S.E. are shown (n = 8–9 each
experiment). Pretreatment of encephalitogenic T cells MBP-TC with Psora-4 (C), charybdotoxin (D), and anan-
damide (E) resulted in an altered disease course. **, p < 0.05.

T cells (0.84 ± 0.15, p = 0.32, n = 5). This could be due to
different binding affinities of the used substances (Fig. 7B). Of
note, the application of channel modulators in vivo was not
associated with obvious side effects. Taken together, results
obtained from the animal model demonstrate the pathophysi-
ological relevance of TASK channel modulation in a T cell-
mediated disorder.

DISCUSSION

Potassium-selective ion channels play a key role in modulat-
ing effector functions of T lymphocytes. Based on convincing
data in vitro and in animal models of autoimmune disorders in vivo, their selective pharmacological intervention has been pro-
posed as a potential therapeutic strategy for T cell-mediated autoimmune disorders (12, 16). Thus far, research reports have
focused on 2 major K+ channels expressed in T cells, (i) the
voltage-dependent Kv1.3 channel and (ii) the Ca2+-activated K
channel KCa3.1. Pharmacological interventions of both types
inhibit T effector functions in vitro and ameliorate disease in
animal models of T cell-mediated autoimmunity in vivo (17).

TASK Channel Expression on Human T Lymphocytes: Implica-
tions for T Cell Effector Functions—Our report identifies and
characterizes the Kp channels TASK1 and -3 as a third K+
conductance relevant for T lymphocyte activation and function. TASK
channel expression was demonstrated by immunocytochemical
stainings as well as Western blotting techniques. Functionally TASK
channels contribute to the membrane resting potential of T cells and
critically affect T cell receptor-mediated effector functions in vitro.
This was evidenced by experiments measuring cytokine secretion and
cell proliferation in the presence of two pharmacological TASK inhibi-
tors and whole cell patch clamp recordings of human CD3+ T cells.
Computational modeling corroborated and extended electrophysi-
ological dissection of the role of TASK channels among three rele-
vant K+ conductances on T cells (Kv1.3, KCa3.1, and TASK) and
support a hypothetical model, how TASK channels contribute to Ca2+
entry and stabilization of the membrane potential. Finally, pathophys-
iological relevance of our findings was demonstrated by testing T cell
selective pharmacological blockade of TASK channels in experimental
autoimmune encephalomyelitis, an animal model of multiple sclerosis:
TASK inhibition was associated with slightly delayed disease onset,
significant amelioration of disease
severity, and significant earlier recovery after adoptive transfer
of pretreated encephalitogenic cells.

Role of Potassium Currents in Ca2+-dependent T Cell
Activation—Interaction of the T cell receptor-CD3 complex
with the antigen-loaded major histocompatibility complex
molecules initiates intracellular signaling via the phospholipase
Cγ pathway, resulting in an initial release of Ca2+ from intra-
cellular stores, increased intracellular Ca2+, activation of pro-
tein kinase C, and augmented expression of Kv1.3 and IK chan-
nels in the cell membrane. Ca2+ re-enters the cell through
CRAC channels, causing membrane depolarization and further
raising intracellular Ca2+ levels. The resulting K+ efflux hyperpolar-
izes the membrane and maximizes the driving force for
continued Ca2+ influx via CRAC channels. T cell response to
antigenic stimulation is highly modulated by the shape and
nature of this calcium signal indicating the importance of dif-
fences in this signaling pattern (16).

The ionic conductances thus far described to be responsible
for calcium signaling in T lymphocytes are the voltage-gated
potassium channel (Kv1.3), the calcium-activated potassium
channel (IKCa1), and CRAC (3, 7, 11, 14, 40, 41). Interestingly,
a broad spectrum of antigenic and mitogenic stimuli of T cells
up-regulate expression of IK channels and also Kv1.3 channels

FIGURE 7. T cell selective modulation of Kv1.3 and Kp attenuates disease in an animal model of multiple sclerosis. A, expression of Kv1.3 (upper row) and TASK1 (lower row) protein in rat MBP-specific T lymphocytes. Left column shows DAPI stainings, right columns the overlay. Representative stainings are shown, scale bar represents 10 μm. B, activated MBP cells pretreated with channel modulators were incubated another 24 h and supernatants were assessed for IFNγ protein levels (un, control cells; C, charybdotoxin 100 nm; P, Psora-4 10 nm; A, anandamide 30 μM). C–E, disease score after transfer of unmanipulated MBP-specific encephalitogenic T cells into Lewis rats is blotted as a solid line. Mean score of all animals including S.E. are shown (n = 8–9 each experiment). Pretreatment of encephalitogenic T cells MBP-TC with Psora-4 (C), charybdotoxin (D), and anan-
damide (E) resulted in an altered disease course. **, p < 0.05.
in vitro (8, 42). The increased expression of the K⁺ channels significantly augments the hyperpolarizing capacity of the activated T cells (compared with naïve cells), accelerates the Ca²⁺ influx, and secures a sustained high level of intracellular Ca²⁺, which is necessary for full-blown T cell proliferation and cytokine production. Accordingly, in the presence of K⁺ blockers, the capability of the T cells to maintain a negative membrane potential and a long-lasting Ca²⁺ signal is reduced, which is the rationale for their putative efficacy in attenuating T cell-mediated immune responses.

Role of K₂P Channels in T Cell Physiology: Electrophysiology and Computational Modeling—What might be the role of our newly described K₂P channels in T cells in this complex interplay? K₂P represent a unique family of potassium channels and are mainly responsible for setting the membrane potential in a number of different cell types (20, 34, 43–46). Given the insensitivity of TASK channels against classical potassium channel blockers, these channels show a response against a unique panel of inhibitors including bupivacaine (23, 47–51), anandamide (35, 52), spermine (53), and ruthenium red (53, 54). Pharmacological interference with TASK channels attenuates T cell proliferation and cytokine secretion, similar to the interference with Kᵥ1.3 using well established channel blocking compounds such as Psora-4 (38) and charybdotoxin (55). Notably, the inhibitory effect on T cell activity was comparable between the Kᵥ1.3 silencing substances Psora-4 and charybdotoxin (note here that charybdotoxin also acts on IKCa1 channels) and the TASK channel inhibitors strongly suggesting an important functional impact of the leak channels on T-cell function.

Contribution of TASK currents to the net outward current of human T lymphocytes was assessed using whole cell patch clamp recordings. A well established protocol stepping the membrane potential from −80 to +40 mV over 500 ms every 30 s elicited a marked outward current with a rapid onset and a slow inactivation (single exponential decay, τ ≈ 195 ms) as described earlier (16). Expectedly, application of Kᵥ1.3 channel modulators resulted in a marked current reduction, whereas current kinetics remained unchanged (16). The endocannabinoid anandamide, however, a well known inhibitor of TASK channels (35, 52), induced a comparable inhibition of the outward current, but in contrast to Kᵥ1.3 blockers anandamide significantly altered current kinetics. Blocker-sensitive currents obtained by graphical subtraction of currents after application of the inhibitor from currents under control conditions showed nearly no voltage dependence in the anandamide-sensitive component, therefore suggesting the contribution of the TASK leak current (23, 34, 35). These findings were corroborated by results from a single-compartment computational model including currents IᵥKᵥ1.3, Iᵥ TASK, and IᵥC. Furthermore, functional expression of KᵥP channels could be shown on isolated human CD3⁺ T lymphocytes and all of the recorded CD3⁺ T cells showed currents indicative of TASK channels. It is currently unknown how (or if) expression of these channels is regulated (e.g. under inflammatory conditions). Furthermore, the investigation of expression levels in different T cell subsets (naïve, Th₁, encephalitogenic T cells, regulatory T cells, CD4⁺, CD8⁺, T central memory cells (Tcm), and T effector memory cells (Tem)) is warranted to determine whether TASK channels correlate to specific T cell functions (similar to Kᵥ1.3 channels). However, based on the abundant expression of the KᵥP channel in a number of different cell types (20, 34, 43–46) it might be speculated that these channels are expressed throughout different T cell subtypes and that influence of TASK on K⁺ conductances represents a “baseline” feature common to all T cells. Our findings imply the possibility that earlier results concerning the net outward current in human T lymphocytes could be contaminated by a TASK channel component, not known or extrapolated at that time. However, the exact role of each component contributing to the outward current in T cell can only be estimated: based on our pharmacological and modeling results we suggest a nearly equal contribution of currents through TASK channels (~40%) and Kᵥ1.3 channels (~40%) to the outward current in human CD3⁺ T cells, whereas other potassium conductances (e.g. IKCa1) contribute to the remaining ~20%. Taken together, our data support the following hypothetical model for KᵥP channels in the cascade of TCR-mediated T cell activation, as summarized in the schematic in Fig. 8.

Selective Blockade of T Lymphocyte TASK Channels Ameliorates Experimental Autoimmune Encephalomyelitis, a Model of Multiple Sclerosis—To prove the pathophysiological relevance of T lymphocyte TASK channels we investigated their modulation in experimental autoimmune encephalomyelitis (27). Selective blockade of T lymphocyte K⁺ channels was achieved by preincubation of encephalitogenic MBP lines prior to transfer with various blockers. This approach was used to dissect the relevance on T cells, which would have been contaminated when applying the channel blockers systemically. In accordance with the literature, Kᵥ1.3 blockade by Psora-4 ameliorated EAE: animals showed a delayed disease onset (nonsignificant), less severe disease course (significant), and a tendency toward
faster recovery (Fig. 7). Importantly, TASK channel modulation via anandamide resulted in very comparable results indicating that TASK-mediated effects on T lymphocytes are of pathophysiologically relevance in a model of T cell-mediated autoimmunity. It is therefore tempting to speculate that attenuation of T cell function by selective pharmacological interference with TASK channels may translate into clinical benefits in T cell-mediated (auto)immune disorders. However, based on the broad expression pattern of TASK channels pharmacological treatment of EAE animals can only partially be linked to TASK channels expressed on immune cells because the effects might be contaminated through effects of TASK channels expressed, e.g., on neurons (13, 23). As a note of caution, long term effects of ion channel blockers inhibiting TASK channels are not known. Therefore further work, including the detailed characterization of TASK1- and TASK3-knock out mice is clearly warranted.

In summary our study introduces TASK channels on T lymphocytes as critical components for the maintenance of resting membrane potential and T cell functions. Accordingly, channel modulation results in a marked reduction of the outward current in human T cells accompanied by significantly altered effector functions. T cell selective pharmacological blockade in an animal model of human multiple sclerosis serves as a proof of concept concerning the (patho)physiological relevance of these channels. Further studies are warranted to delineate the potential of TASK channels as a target and TASK channel blockers as potential drug candidates in T cell-mediated autoimmune diseases such as multiple sclerosis.

Acknowledgments—We thank Astrid Schmitt and Sabrina Braun-schweig for excellent technical assistance.

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