**KCNE gene expression is dependent on the proliferation and mode of activation of leukocytes**

Laura Solé,1 Albert Vallejo-Gracia,1 Sara R. Roig,1 Antonio Serrano-Albarrás,1 Laura Marruecos,2 Joan Manils,2 Diana Gómez,2 Concepció Soler2 and Antonio Felipe1,*

1Molecular Physiology Laboratory; Departament de Bioquímica i Biologia Molecular; Institut de Biomedicina; Universitat de Barcelona; Barcelona, Spain; 2Departament de Patologia i Terapèutica Experimental; Facultat de Medicina; Campus de Bellvitge; Universitat de Barcelona; L’Hospital de Llobregat; Barcelona, Spain

**Keywords**: voltage-dependent potassium channels, gene expression, macrophages, lymphocytes, cell-cycle, activation, regulatory subunits

Voltage-dependent K⁺ (Kv) channels are tightly regulated during the immune system response. Leukocytes have a limited repertoire of Kv channels, whose physiological role is under intense investigation. A functional Kv channel is an oligomeric complex composed of pore-forming and ancillary subunits. The KCNE gene family is a novel group of modulatory Kv channel elements in leukocytes. Here, we characterized the gene expression of KCNEs (1–5) in leukocytes and investigated their regulation during leukocyte proliferation and mode of activation. Murine bone-marrow-derived macrophages, human Jurkat T-lymphocytes and human Raji B-cells were analyzed. KCNEs (1–5) are expressed in all leukocytes lineages. Most KCNE mRNAs show cell cycle-dependent regulation and are differentially regulated under specific insults. Our results further suggest a new and yet undefined physiological role for KCNE subunits in the immune system. Putative associations of these ancillary proteins with Kv channels would yield a wide variety of biophysically and pharmacologically distinct channels that fine-tune the immunological response.

Introduction

Voltage-dependent potassium (Kv) channels play an important role in determining resting membrane potential and controlling action potentials in nerves and muscles.1 In addition, Kv channels control the proliferation and activation of macrophages and lymphocytes.2,3 Leukocytes have a limited repertoire of Kv channels, which include Kv1.3, Kv1.5 and Kv11.1, which are major determinants of cell physiology.4 While Kv1.3 is ubiquitous in the immune system, Kv1.5 functions primarily in mononuclear phagocytes and Kv11.1 in lymphoblastoid proliferative cells.5-8 In addition, other Kv channels play minor roles and have also been detected in specific cell subtypes.9,10 Many Kv channels can assemble promiscuously, yielding a wide variety of biophysically and pharmacologically distinct channels. Therefore, native currents are not necessarily recapitulated through the activity of a single subunit. Furthermore, the presence of regulatory subunits enhances this complexity.11

Kvβ subunits with Kv1.3 and Kv1.5 channels generates distinct functional Kv channel complexes that fine-tune the cellular response.11-13 There has been a massive effort by the research community to characterize Kv channels; however, there are few studies concerning regulatory subunits in leukocytes.11-13 The recent identification of novel modulatory proteins has attracted much attention.

KCNEs are a group of regulatory subunits composed of five members (KCNE1–5).14 KCNE peptides are single-span membrane proteins that modulate many Kv channels. Characterized interactions, such as Kv7.1 (KCNQ1)/KCNE1 and Kv11.1 (hERG)/KCNE2 channels, recapitulate the cardiac Iks and Ikr currents, respectively.14 Several KCNE mutations trigger severe cardiac channelopathies and demonstrate the pivotal role of these ancillary peptides in cardiovascular physiology. The implication of KCNE1–5 peptides in cardiac physiology is under intense investigation.15 Although many KCNE subunits share tissue expression with Kv channels, their interactions need to be defined. Thus, Kv1.3 and KCNE1 were simultaneously cloned in human Jurkat T-cells.16 It was shown that KCNE4 modulates Kv1.1 and Kv1.3 channels and their heteromeric forms.17 Furthermore, an interaction between KCNE2 with Kv1.5 and Kv1.3 in murine ventricles and choroid plexus epithelium has been proposed.18,19

*Correspondence to: Antonio Felipe; Email: afelipe@ub.edu
Submitted: 10/05/12; Revised: 12/13/12; Accepted: 12/13/12
http://dx.doi.org/10.4161/chan.23258

©2013 Landes Bioscience. Do not distribute.
Scarce information is available concerning the role of KCNE peptides in leukocytes. We recently demonstrated that KCNE4 associates with Kv1.3, suppressing K⁺ currents through modulations in trafficking, surface expression and channel gating. Although few preliminary screenings support a KCNE variable phenotype, there have been no KCNE regulation studies involving the immune system. Therefore, the aim of the present work was to identify the presence of KCNE regulatory subunits in leukocytes and further characterize their expression during leukocyte proliferation and activation. We demonstrate that macrophages and T- and B-lymphocytes express KCNE subunits. In addition, the expression of many KCNE proteins is cell cycle dependent, and distinct cell-specific insults differentially regulate KCNE expression. In summary, leukocytes express KCNE regulatory subunits, and their expression varies with proliferation and activation; therefore, we suggest that the heteromeric Kᵥ channel structure might be variable.

**Results**

Regulation of KCNE modulatory subunits through MCSF-dependent proliferation and LPS- and IFNγ-induced activation in macrophages. Macrophages perform critical functions in the immune system, acting as regulators of homeostasis and effector cells in infection, wounding, and tumor growth. In response to different growth factors and cytokines, macrophages proliferate and become activated or differentiated. We used BMDM as a cell model that mimics the physiological schedule within the body. Mature cells might proliferate or become activated after the application of specific stimuli, such as MCSF, which is a specific growth factor for this cell type. After incubation of BMDMs for 18–24 h in the absence of MCSF, cell growth was terminated (> 98%) and the cells became quiescent. The addition of MCSF (1200 U/ml) triggered macrophage proliferation and an increase of the outward K⁺ currents (Fig. 1A and Fig. S1). Although BMDMs expressed all five KCNE subunits, different cell cycle-dependent patterns were observed (Fig. 1B–G). KCNE2, KCNE4 and KCNE5 notably increased at 6 h during the early phases (G1/S) of the cell cycle (Fig. 1C, E and F). In contrast, KCNE3 exhibited the opposite behavior (Fig. 1D), and KCNE1 levels remained constant throughout the cell cycle (Fig. 1B). Therefore, proliferation generated major differences in the KCNE phenotype (Fig. 1G).

The incubation of bone marrow macrophages with LPS and IFN-γ inhibits cell proliferation. However, while LPS triggers apoptosis, IFN-γ protects the cells from programmed cell death. Figure 2 shows that after 24 h of IFN-γ-induced activation (300 U/ml), confirmed by iNOS abundance (Fig. 2A), the expression of the KCNE subunits was relatively stable. Moreover, a gradual decrease of KCNE3 expression was also observed (Fig. 2D), while the other KCNEs experienced only minor changes in expression (Fig. 2B–F). Similar to proliferation, KCNE4 and KCNE5 were the most abundant subunits, and KCNE3 was the least abundant subunit (Fig. 2G).

The addition of 100 ng/ml LPS to proliferating macrophages elevated K⁺ currents (Fig. S1), induced the expression of iNOS (Fig. 3A), blocks the cell cycle at the G1/S boundary and induces apoptosis. Under this treatment, the expression of not only KCNE3 but also KCNE4 and apparently, but not significantly, KCNE2 was also reduced (Fig. 3B–F). However, the KCNE phenotype in LPS-treated macrophages was similar to that observed with IFN-γ. Thus, KCNE4 and KCNE5 were the most abundant subunits, whereas KCNE3 was least abundant (Fig. 3G).

**KCNE regulatory subunits during the proliferation and LPS- and PMA-induced activation of Raji B-lymphocytes.** Human B-lymphocytes express Kᵥ1.3 and Kᵥ11.1 channels that might be associated with KCNE subunits. Raji B lymphocytes are a lymphoblast-like cell line derived from Burkitt’s B-cell lymphoma. Although neoplastic proliferation increases the expression of Kᵥ1.3 and Kᵥ11.1, no information is available concerning the KCNE subunits. Unlike macrophages, activated B cells undergo proliferation and differentiation into memory and plasma cells. We first analyzed the expression of KCNE subunits in resting Raji B-lymphocytes. The abundance of KCNE subunits in B-cells starved for 24 h indicated that the expression of KCNE1 and KCNE4 was extremely low (Fig. 4G). FBS-dependent proliferation triggered the differential regulation of KCNE subunits (Fig. 4A–F). While KCNE2, KCNE3 and, to a lesser extent, KCNE4 showed decreased expression after 24 h, the expression of KCNE1 and KCNE5 remained constant (Fig. 4B–F). However, the KCNE profile in B cells throughout the cell cycle progression was mostly stable and after 24 h, the KCNE phenotype was recovered to that observed at the initial stage (Fig. 4G).

Raji B-lymphocytes, incubated with LPS (100 μg/ml), became activated as confirmed by the expression of TNFα (Fig. 5A). However, after 24 h of LPS-induced activation, no major changes in the KCNE profile were observed (Fig. 5B–G). In addition, 10 nM PMA activation, which also elevated TNFα expression (Fig. 6A), did not trigger apparent changes in KCNE expression (Fig. 6B–F). As a consequence, and similar to the results obtained with LPS-induced activation, no major differences in the KCNE profile were observed (Fig. 6G).

**KCNE regulatory subunits during the proliferation and PMA/PHA-induced activation of Jurkat T-lymphocytes.** Human T-lymphocytes expressing a limited repertoire of Kᵥ1 channels, which include Kᵥ1.1, Kᵥ1.4, Kᵥ1.6 and Kᵥ1.3, are Kᵥ1.3 and Kᵥ11.1, were isolated simultaneously in Jurkat T cells, and KCNE4 regulates Kᵥ1.1 and Kᵥ1.3,17,20 Although we observed the expression of all KCNE transcripts in Jurkat T cells, surprisingly, KCNE1 was, by far, the least abundant KCNE subunit in Jurkat cells (Fig. 7G). Unlike KCNE1, KCNE3 and KCNE5, FBS-dependent proliferation (Fig. 7A) triggered a cell cycle-dependent increase of KCNE2 and KCNE4 expression (Fig. 7B–F). Compared with the relative abundance among KCNE subunits under FBS-dependent proliferation, the higher increase corresponded with KCNE4 expression. However, the KCNE profile experienced minor changes (Fig. 7G).

The combination of PHA (5 μg/ml) and PMA (80 nM) triggers an important activation of Jurkat T cells reflected by the massive production of IL-2 (Fig. 8A). Under these circumstances, an important remodeling of KCNE mRNA expression.
was observed (Fig. 8B–G). Again, KCNE1, the less abundant subunit, remained constant (Fig. 8B and G). However, the KCNE4 mRNA expression, which was initially low, showed an increase under PHA/PMA incubation (Fig. 8E and G). Moreover, the expression of KCNE5 and, to a lesser extent, KCNE3 steadily increased (Fig. 8D and F). In contrast, KCNE2 expression was significantly decreased (Fig. 8C). Our results demonstrated that the expression of KCNE4 was, by far, the major regulated under PMA/PHA-induced activation (Fig. 8G).

Discussion

Kv channels are involved in maintaining resting membrane potential and participate in the immune system response. In nerve and muscle cells, native K+ currents are not recapitulated through single-channel genes. Therefore, the hetero-oligomerization of related Kv subunits and their association with regulatory peptides to form functional channelosomes is crucial for cell physiology. Leukocytes have a limited Kv repertoire that remains elusive. Kv channels in the immune system might be formed through associations between Kv products and auxiliary subunits, which enormously increase the K+ current phenotype and fine-tune the immunological response. Thus, Kv1.3 and Kv1.5, and Kv11.1 channels have been implicated as important targets in leukocyte physiology. In addition, Kv1.1, Kv1.2, Kv1.6, Kv3.1 and Kv7.1 have also been detected in

Figure 1. Expression and regulation of KCNE (1–5) mRNA in MCSF-dependent BMDM proliferation. G0-arrested BMDMs were incubated in the presence of 1,200 U/ml MCSF, and the mRNA was isolated simultaneously after the indicated times of treatment. Real-time PCR was performed as described in Materials and Methods. (A) MCSF-dependent proliferation of BMDM. The values are given as the mean ± SEM and represent cell number of four independent experiments each performed in triplicate. ○, +MCSF; ●, −MCSF. (B–F) Relative mRNA expression, in arbitrary units (AU), of KCNE1–5. (B) KCNE1; (C) KCNE2; (D) KCNE3; (E) KCNE4; (F) KCNE5. The values are given as the mean ± SEM (n = 3–6). *, p < 0.05; **, p < 0.01 (Student’s t-test). (G) KCNEx/KCNE1 ratio. The relative expression of each KCNE and the KCNEx/KCNE1 ratio was calculated using the KCNE specific real-time PCR efficiency. See Materials and Methods for details. White bars, 0 h; gray bars, 6 h; black bars, 24 h of MCSF-dependent proliferation.
specific leukocytes. The oligomerization of several of these subunits might contribute to the diversity of K+ currents, but the participation of modulatory subunits must also be considered. Regulatory Kv subunits are primarily distributed into three main groups: (1) the Kvβ subunit gene family, with three major gene products, Kvβ1, Kvβ2, and Kvβ3; (2) the KCNE family, which comprises five different members (KCNE1–5); and (3) the potassium channel-interacting (KChip) and potassium channel-accessory (KChap) proteins, which form an heterogeneous group of peptides.

While the last group is primarily considered as a collection of chaperones, which almost exclusively modulate the level of channel surface expression, the former groups also control kinetics. There is a considerable amount of data concerning the Kvβ interaction with Kv channels and the identification of these subunits in leukocytes. However, the presence and the regulation of KCNE subunits in the immune system have not been thoroughly addressed, although sound evidence indicates that KCNE peptides might modulate leukocytic Kv channels.

The present work is the first to systematically analyze the expression of KCNE1–5 mRNA in differentiated leukocytes, such as macrophages and B and T lymphocytes. Although all leukocytes expressed KCNE1–5, the mRNA abundance varied among the cell lineage. Preliminary and heterogeneous affymetrix gene chip studies have previously addressed this issue leading to controversial interpretations as discussed below (see Supplemental Material for details). In this context, our study paves the way for further research. However,
our work relies on the KCNE gene expression and possible post-transcriptional modifications should be contemplated for future research.

Because Kv channels play crucial roles in the immune response, we further studied KCNE mRNA regulation upon leukocyte proliferation and activation. A few studies have documented the expression of KCNE subunits in the immune system, but show contradictory results. In 1992, Attali and coworkers isolated KCNE1 from Jurkat T cells, but reported no changes upon activation.16 Chouabe et al. (1997) confirmed the expression of KCNE1 in peripheral blood leukocytes but observed no expression in the spleen and thymus.34 The results of these pioneering studies have led to controversial debates concerning which cell lineage expresses KCNE1, and what state of differentiation/maturation/activation is required to detect KCNE1 expression. Here, we further confirmed that KCNE1 expression, which is scarcely detected, remained almost consistent upon proliferation or under various insults in macrophages and B and T lymphocytes. Our results are consistent with those of Lunquist et al. (2006) who demonstrated that the KCNE1 mRNA is the least abundant subunit in thymus, spleen and peripheral leukocytes.35 However, the array data from different sources support contradictory results, arguing that there is similar mRNA expression among the KCNEs, with no changes in neoplastic growth between the lineages (GeneChips from GeneNote, http://bioinfo2.weizmann.ac.il/cgi-bin/genenote/home_page.pl and GNF BioGPS, http://biogps.gnf.org, see Supplemental Material for extended information). This could be characteristic of the immune system because the upregulation of KCNE1 has been previously shown under neoplastic growth in other tissues.36,37 Similar differential tissue-specific responses have also been demonstrated with Kv1.3 and Kv1.5 in macrophages and muscle cells.3,38
Are there other KCNEs expressed in the immune system? The expression of other KCNEs has not been thoroughly examined in leukocytes. The expression of KCNE2 in leukocytes and tissues from the immune system is limited. We observed that MCSF and FBS induce a notable cell cycle-dependent upregulation of KCNE2 mRNA. However, affymetrix GeneChip studies show that KCNE2 mRNA abundance is similar between cancerous and healthy thymus, bone marrow and whole blood specimens. In contrast, activation triggers the downregulation of KCNEs in Jurkat T cells, but other insults failed in macrophages or Raji B cells. The results obtained from array data from experiments performed in different leukocytic, human leukemia and lymphoma cell lines suggest that KCNE2 expression is quite stable (see Supplemental Material for GeneChip information). Changes during the cell cycle and/or differentiation might explain these differences. In fact, in most cases, we report that the upregulation of KCNE2 was transient and returned to basal levels during late phases of the cell cycle.

The expression of KCNE3 presents an interesting debate. While Abbott et al. (2001) reported no expression of KCNE3 in thymus, spleen and human leukocytes, Lundquist et al. (2006) observed the abundant expression of KCNE3 in the same tissues. Other evidence also indicates a disparity among cell lines. For instance, array data from GNF BioGPS has shown that KCNE3 might be prominent in cells of a myeloid origin (see Supplemental Material for GeneChip information). Our data do not support the results obtained from the last study because KCNE3 expression was low.
in macrophages. However, KCNE3 mRNA suffers differential regulation between proliferation and activation with a remarkable downregulation under LPS and IFN-γ incubation. Furthermore, we did observe notable KCNE3 mRNA expression in human B and T lymphocytes, with minor regulation upon proliferation or activation.

Grunnet et al. (2003) documented the consistent expression of KCNE4 in activated T lymphocytes. KCNE4 expression is relatively abundant in the immune system (see supplemental material for GeneChip information). Although most studies show that KCNE4 mRNA levels are slightly higher than other KCNEs in the spleen, multiple native leukocytes and several leukemia and lymphoma cell lines show little expression in peripheral leukocytes. Our data support the idea that the mRNA levels of KCNE4 are quite abundant, and experienced interesting regulation. We have previously reported that while KCNE4 expression increased in LPS-activated Raw 264.7 macrophages, the immunosuppressant dexamethasone triggered no changes. In the present work, we further documented the cell cycle-dependent upregulation of KCNE4 in BMDM, whereas the levels of mRNA remained constant or decreased upon IFN-γ and LPS incubation, respectively. Unlike in Raw cells, the presence of LPS and IFN-γ inhibits proliferation in BMDMs. IFN-γ and LPS arrests cells at the G1/S boundary, but only LPS induces apoptosis. Thus, Kv1.3, a documented target of KCNE4-dependent negative regulation, potentially plays a dual role in proliferation and apoptosis. Therefore, it is tempting to speculate that the Kv1.3 activity should be conserved during proliferation and apoptosis in BMDMs. We observed similar regulation in T lymphocytes but not in B cells, where KCNE4 mRNA levels remained consistent.

Finally, KCNE5 is the less documented KCNE. The results of affymetrix GeneChip studies (see Supplemental Material for GeneChip information) have demonstrated that KCNE5 is present in cells and healthy and cancerous tissues from the immune system. However, no KCNE5 regulation has been documented. We observed the cell cycle-dependent regulation of KCNE5 in BMDMs and a steady increase in activated T-lymphocytes. In general, our results would support quite notable levels of KCNE5

Figure 5. Expression and regulation of KCNE (1–5) mRNA in LPS-activated Raji B lymphocytes. B cells were incubated in the presence of LPS (100 μg/ml), and the mRNA was isolated simultaneously after the indicated times of treatment. Real-time PCR was performed as described in Materials and Methods. (A) TNFα mRNA expression. Ribosomal 18S RNA was used as control. (B–F) Relative mRNA expression, in arbitrary units (AU), of KCNE1–5. (B) KCNE1; (C) KCNE2; (D) KCNE3; (E) KCNE4; (F) KCNE5. The values are given as the mean ± SEM (n = 3–6). (G) KCNEx/KCNE1 ratio. The relative expression of each KCNE and the KCNEx/KCNE1 ratio was calculated using the KCNE specific real-time PCR efficiency. See Materials and Methods for details. White bars, 0 h; gray bars, 6 h; black bars, 24 h in the presence of LPS.

A  LPS (h)  0  6  24

B  KCNE1

C  KCNE2

D  KCNE3

E  KCNE4

F  KCNE5

G  KCNEx/KCNE1 ratio (log)

0 h  6 h  24 h

LPS activation time
mRNA in leukocytes consistent with the highest levels detected in thymus and myeloid cells (see Supplemental Material for GeneChip information). In summary, our work systematically addresses, for the first time, the mRNA expression of the five KCNE subunits in leukocytes. These newly identified auxiliary leukocytic channel subunits might exert regulatory actions, such as the regulation of Kv gating, traffic and localization. This field is in its infancy and these data serve as a useful roadmap to inform future studies and represents a step toward understanding the role of these regulatory subunits in leukocyte physiology.

**Materials and Methods**

**Animals, cell culture and proliferation assays.** Murine bone marrow-derived macrophages (BMDM) from 6- to 10-wk-old BALB/c (Charles River laboratories) were used. The cells were isolated and cultured as described elsewhere. Briefly, animals were sacrificed by cervical dislocation, and the adherent tissue was removed to dissect both femurs. The ends of bones were cut off, and the marrow tissue was flushed using irrigation with medium. The marrow plugs were passed through a 25-gauge needle for dispersion. The cells were cultured in DMEM containing 20% FBS and 30% L-929 fibroblast (L-cell) conditioned media as a source of macrophage colony-stimulating factor (MCSF). The macrophages were obtained as a homogeneous population of adherent cells after 7 d of culture and maintained at 37°C in a humidified incubator under 5% CO₂. For the experiments, the cells were cultured with tissue culture differentiation medium (DMEM, 20% FBS, 30% L-cell medium) or arrested at G₀ using MCSF deprivation in DMEM supplemented with 10% FBS for at least 18 h. The G₀-arrested cells were further incubated in the presence of 10 nM PMA and mRNA was isolated simultaneously after the indicated times of treatment. Real-time PCR was performed as described in Materials and Methods.
Raji human B lymphocytes and Jurkat human T lymphocytes were routinely cultured in RPMI culture media containing 10% FBS and supplemented with 10 U/mL penicillin and streptomycin. In proliferation experiments, the Raji B cells and Jurkat T cells were incubated for 24 h in RPMI media without FBS supplemented with 0.02% BSA (G₀-arrested cells) prior to culture in regular RPMI supplemented with 10% FBS. While 10 nM phorbol ester (PMA, Sigma-Aldrich) and LPS (100 μg/ml) were used to activate B lymphocytes, the Jurkat T cells were stimulated with 5 μg/ml of phytohemagglutinin (PHA, Sigma-Aldrich) supplemented with 80 nM of PMA. Control cells treated with DMSO, the vehicle for PMA, were used to discard possible non-specific side effects.

Proliferation was investigated by analyzing the cell number and viability. A Countess™ automated cell counter (Invitrogen) was used for cell counting and viability analyzed by means of tripan blue exclusion. Viabilities higher than 85 ± 15% were used in all groups.

RNA isolation, RT-PCR analysis and real-time PCR. Cells cultured in 100-mm tissue culture dishes were incubated for a 24 h test period before harvesting, with agents added 6 or 24 h before harvesting or with no agents added (“0 h,” see Fig. S2). Total RNA from BMDM, Raji B cells and Jurkat T cells was isolated using the Nucleospin RNA II kit (Invitrogen), which contains DNase I. PCR controls were performed in the absence of reverse transcriptase. cDNA synthesis was performed using Transcriptor Reverse Transcriptase (Roche) with a random hexanucleotide and oligo(dT)

Figure 7. Expression and regulation of KCNE (1–5) mRNA in FBS-dependent Jurkat T-lymphocyte proliferation. G₀-arrested T cells were incubated in the presence of FBS, and the mRNA was isolated simultaneously after the indicated times of treatment. Real-time PCR was performed as described in Materials and Methods. (A) FBS-dependent proliferation of Jurkat T-cells. The values are given as the mean ± SEM and represent cell number of four independent experiments each performed in triplicate. ●, + FBS; ○, - FBS. (B–F) Relative mRNA expression, in arbitrary units (AU), of KCNE1–5. (B) KCNE1; (C) KCNE2; (D) KCNE3; (E) KCNE4; (F) KCNE5. The values are given as the mean ± SEM (n = 3–6). *, p < 0.05; **, p < 0.01 (ANOVA and post-hoc Tukey’s test). (G) KCNEx/KCNE1 ratio. The relative expression of each KCNE and the KCNEx/KCNE1 ratio was calculated using the KCNE specific real-time PCR efficiency. See Materials and Methods for details. White bars, 0 h; gray bars, 6 h; black bars, 24 h of FBS-dependent proliferation.
primers according to the manufacturer’s instructions. Real-time PCR was performed using a thermocycler (Applied Biosystems) and the LightCycler FastStart DNA MasterPLUS SYBR Green I kit (Roche) according to the manufacturer’s instructions. The PCR primer sequences, gene accession numbers, annealing conditions and amplicon lengths for each KCNE are listed in Table 1. The reactions were performed under the following conditions: an initial incubation at 95°C for 10 min followed by cycling at 95°C for 5 sec, specific annealing at T°C for 8 sec, and 72°C for 6–9 sec, and a final incubation at 95°C for 10 min. Melting curves were performed to verify the specificity of the product, and ribosomal 18S RNA was included as an internal reference as previously described.3,12 The results were analyzed using commercial software (Applied Biosystems). For each primer set, a standard curve was generated, and the slope factor was calculated. The values were normalized to the corresponding 18S RNA. The corresponding real-time PCR efficiency (E) of one cycle in the exponential phase was calculated according to the equation

\[ E = 10^{(-1/\text{slope})}. \]

The normalized KCNEx/KCNE1 ratio was calculated taking into account the primer pair efficiencies as follows: Ratio = (1+E) \( \Delta C_t \) (KCNEx)/ (1+E) \( \Delta C_t \) (KCNE1), where Ct signifies the threshold cycle.41

**Northern blot analysis.** Up to 20 μg of total RNA was fractionated by electrophoresis through a 1% agarose, 3% formaldehyde gel in 20 mm MOPS and 1 mm EDTA, pH 7.4. Application of equal amounts of RNA to each lane was confirmed by the addition of ethidium bromide to the samples before electrophoresis. RNA was transferred overnight to an Immobilon filter (Amersham Pharmacia Biotech) by capillary action in 20 × SSC (SSC, 3 min NaCl, 300 mm sodium citrate, pH 7.0). RNA was cross-linked to the filter by irradiation with UV light. Filters were hybridized with hTNF-α and rat 18S rRNA cDNA probes. Filters were washed once for 30 min at 65°C in 3 × SSC and 1% lauryl sulfate, once in 1 × SSC and 1% lauryl sulfate, and once with 0.2 × SSC and 1% lauryl sulfate before autoradiography.
**Statistics.** The values are expressed as the mean ± SEM of 3–6 experiments performed in triplicate. The significance of the differences was established using Student’s t-test or one-way ANOVA followed by a post-hoc Tukey’s test (GraphPad, PRISM 5.0) where indicated. The decision to perform one-way ANOVA analysis was considered when significance was not observed using Student’s t test. A value of p < 0.05 was considered significant. Due to the mathematical transformation, the statistical significance of the normalized KCNEx/KCNE1 ratio was considered irrelevant. The data should be considered qualitatively illustrative rather than a quantitative value.

**Disclosure of Potential Conflicts of Interest**
No potential conflicts of interest were disclosed.

**Acknowledgments**
Supported by the Ministerio de Economia y Competitividad (MINECO), Spain (BFU2011-23268 and CSD2008-00005 to A.F., BFU2010–15674 to C.S.), L.S., A.V.G., S.R.R. and D.G. hold fellowships from the MINECO. J.M. was an APIF fellow (Universitat de Barcelona). L.S. and A.V.G. contributed equally.

**Supplemental Materials**
Supplemental materials may be found here: www.landesbioscience.com/journals/chan/article/23258
13. 11. 10. 9. 7. 5. 4. 3. 2. 1.

Chem 1999; 274:20123-6; PMID:10400624; http://pmid:16039274; http://dx.doi.org/10.1016/j.cardio-

11. Tamkun MM, et al. Pattern of Kv beta subunit expres-

sion in macrophages depends upon proliferation and

10. some 11, encodes the type l K+ channel in T cells. J Biol

9. Wasmuth JJ, Gutman GA, et al. The Shaw-related

7. potassium channel gene, Kv3.1, on human chromo-

5. Tsui FW, et al. Functional up-regulation of HERG K+

4. A, Guasti L, et al. HERG potassium channels are con-

3. expression and function by TCR and costimulatory sig-

2. Tsui HW, et al. Functional up-regulation of HERG K+

1. some 11, encodes the type l K+ channel in T cells. J Biol

References

Hille B. Ion channels of excitable membranes. Sunderland, Mass.; [Great Britain]: Sinauer, 2001.

1. Conforti L. The ion channel network in T lympho-

41. Villalonga N, David M, González T, Villalóna N, David M, Marionneau C, Haurogne K, Larroque

39. Norris AJ, Fojer NC, Kerbein JM. Neuronal voltage-gated K+ (Kv) channels function in macro-

37. Pourrier M, Schram G, Nattel S. Properties, expression and potential roles of cardiac K+ channel acces-

35. Pourrier M, Schram G, Nattel S. Properties, expression and potential roles of cardiac K+ channel acces-

33. Pourrier M, Schram G, Nattel S. Properties, expression and potential roles of cardiac K+ channel acces-

31. Pastore-Anglada M. Regulation of nucleoside trans-

29. David M, Macías A, Moreno C, Prieto A, MartíNEz-MárMol R, Vicente R, et al. Protein kinase C (PKC)

27. Pauls TJ, Vicente R, Grande M, López-Iglesias C, Figuera C, Capella G, et al. KCNQ1/KCNKE chan-

25. Tilló E, López-Iglesias C, et al. Differential voltage-

23. Smith GA, Tsu HW, Newell EW, Jiang X, Zhu XP, Tsu HW, et al. The functional and structural prop-

21. Liu QH, Fleischmann BK, Hondowicz B, Maier CC, Beeton C, Pennington M, Cahalan MD, Chandy K G. The functional network

19. Smith GA, Tsu HW, Newell EW, Jiang X, Zhu XP, Tsu HW, et al. The functional and structural prop-

17. Tsuei FW, et al. Pattern of Kv beta subunit expres-

15. Bendahlou S, Marionneau C, Haurogne K, Larroque MM, Derand R, Snau V, et al. In vitro molecular interac-

13. 11. 10. 9. 7. 5. 4. 3. 2. 1.

11. Tamkun MM, et al. Pattern of Kv beta subunit expres-

9. Wasmuth JJ, Gutman GA, et al. The Shaw-related

7. potassium channel gene, Kv3.1, on human chromo-

5. Tsui FW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol

5. Tsui HW, et al. Functional up-regulation of HERG K+

3. expression and function by TCR and costimulatory sig-

1. some 11, encodes the type l K+ channel in T cells. J Biol