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Altered Dynamics of Kv1.3 Channel Compartmentalization in the Immunological Synapse in Systemic Lupus Erythematosus

Stella A. Nicolaou,* Peter SzligiGETi, Lisa Neumeier, Susan Molleran Lee,† Heather J. Duncan,* Shashi K. Kant,* Anne Barbara Mongey,* Alexandra H. Filipovich,‡ and Laura Conforti2*‡

Aberrant T cell responses during T cell activation and immunological synapse (IS) formation have been described in systemic lupus erythematosus (SLE). Kv1.3 potassium channels are expressed in T cells where they compartmentalize at the IS and play a key role in T cell activation by modulating Ca\(^2+\) influx. Although Kv1.3 channels have such an important role in T cell function, their potential involvement in the etiology and progression of SLE remains unknown. This study compares the K channel phenotype and the dynamics of Kv1.3 compartmentalization in the IS of normal and SLE human T cells. IS formation was induced by 1–30 min exposure to either anti-CD3/CD28 Ab-coated beads or EBV-infected B cells. We found that although the level of Kv1.3 channel expression and their activity in SLE T cells is similar to normal resting T cells, the kinetics of Kv1.3 compartmentalization in the IS are markedly different. In healthy resting T cells, Kv1.3 channels are progressively recruited and maintained in the IS for at least 30 min from synapse formation. In contrast, SLE, but not rheumatoid arthritis, T cells show faster kinetics with maximum Kv1.3 recruitment at 1 min and movement out of the IS by 15 min after activation. These kinetics resemble preactivated healthy T cells, but the K channel phenotype of SLE T cells is identical to resting T cells, where Kv1.3 constitutes the dominant K conductance. The defective temporal and spatial Kv1.3 distribution that we observed may contribute to the abnormal functions of SLE T cells.


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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; [Ca\(^2+\)]\(_i\), intracellular Ca\(^2+\) level; Tcm, central memory T cell; Tem, effector memory T cell; IS, immunological synapse; PKC, protein kinase C; SLEDAI, SLE disease activity index; RA, rheumatoid arthritis; SEB, staphylococcal enterotoxin B; MFR, mean fluorescent ratio; HP, holding potential; EC, extracellular; IC, intracellular; C, Caucasian; AA, African American.

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components (e.g., Lck and protein kinase Cθ (PKCθ)) (18). Functionally, the process of IS formation is thought to facilitate signaling through the TCR and to fine-tune the ultimate outcome of TCR engagement. The structure of the IS is very dynamic, with molecules entering and leaving at different times. However, the process of Kv1.3 channel relocalization in the IS is not yet understood.

Furthermore, no information is available on potential alterations in Kv1.3 channel redistribution at the IS in pathological conditions. SLE T cells display certain features that can affect the formation of the IS: SLE T cells possess greater capacity to generate lipid rafts than normal T cells in response to activation, faster kinetics of lipid raft clustering and polarization, and faster kinetics of actin polymerization and depolymerization (6). In particular, it has been shown that cross-linking of lipid rafts evokes faster and more pronounced Ca\(^{2+}\) response in SLE T cells, indicating that early structural rearrangements in the T cell membrane contribute to the increased activity of SLE T cells.

The purpose of our study was to investigate whether the expression and activity of key regulators of Ca\(^{2+}\) homeostasis, such as Kv1.3 channels, are altered in SLE T cells. Furthermore, we have investigated whether abnormalities in the process of translocation of these channels in the IS that forms upon TCR binding occur in SLE T cells. Our results indicate that while the biophysical and pharmacological properties of Kv1.3 channels in SLE T cells are identical to normal T cells, the dynamics of Kv1.3 channel compartmentalization in the IS of SLE T cells are altered. These alterations in TCR-activated membrane rearrangements might underlie the downstream functional abnormalities of SLE T cells.

**Human subjects**

Twenty patients with SLE fulfilling at least four of the American College of Rheumatology classification criteria for SLE were included in this study: 3 males and 17 females, 4 Caucasian (C), 14 African American (AA), and 2 Hispanic, age 24–68 years (19, 20). Eleven had lupus nephritis, of whom 2 required dialysis. In our cohort, 17 patients had a SLE disease activity index (SLEDAI) >3, indicative of active disease, and 18 were being treated with immunosuppressive therapy (21). Control groups consisted of 5 patients with rheumatoid arthritis (RA) who fulfilled the American College of Rheumatology classification criteria for RA and 26 healthy individuals. The RA group consisted of 5 females, 2 C and 3 AA, with an age range of 40–68 years. The healthy control group consisted of 6 males, 17 females, and 3 unknown. 21 C, 2 AA, and 3 unknown, age 30–54 years. The study was approved by the University of Cincinnati Institutional Review Board.

**Cells**

PBMC, CD3\(^{+}\), CD4\(^{+}\), and CD8\(^{+}\) lymphocytes were isolated from venous blood collected from consenting donors by Ficoll-Paque density gradient centrifugation (ICN Biomedicals) and E-rosetting (StemCell Technologies) as previously described (22). The homogeneity of the T cell populations was determined by FACS (22). Cells were maintained in RPMI 1640 medium supplemented with 10% pooled human AB serum (Invitrogen Life Technologies), 200 U/ml penicillin, 200 µg/ml streptomycin, 1 mM HEPES. Preactivated T cells were obtained by exposure to 4\(\mu\)g/ml staphyloccocal enterotoxin B (SEB; Sigma-Aldrich), for 2 h at 37°C and labeled with 5 µM cell tracker blue CMAC (Molecular Probes). T cells were mixed with either beads or B cells at a ratio of 1:1.5 and spun briefly at 100 g. After stimulation, they were maintained in a humidified incubator at 37°C for 1–30 min and plated onto poly-L-lysine-coated coverslips. Attached cells were fixed with 4% paraformaldehyde for 20 min, blocked using 10% normal goat serum or horse serum, permeabilized with 0.2% Triton X-100, and incubated overnight with primary Abs followed by the appropriate fluorescent secondary Abs (Molecular Probes). The primary Abs used for detecting Kv1.3 proteins were either a rabbit polyclonal anti-Kv1.3 Ab against an epitope on the C-terminal domain of the protein (Alomone) or an extracellular (EC) epitope (Sigma-Aldrich). The latter was used for labeling “live” Kv1.3 channels in T lymphocytes before interaction with the EBV-B cells. F-actin and GM1 were stained using Alexa Fluor 546 phalloidin and Alexa Fluor 555 cholera toxin B, respectively (Molecular Probes) and CD3\(^{+}\) was stained with a goat anti-CD3e Ab (Santa Cruz Biotechnology).

**Electrophysiology**

Kv1.3 currents were recorded in whole cell configuration. The external solution for activating and recording KCa3.1 currents had the following composition (in millimoles): 160 NaCl, 4.5 KCl, 2.0 CaCl\(_2\), 1.0 MgCl\(_2\), and 10 HEPES (pH 7.4). The pipette solution was composed of (in millimoles): 145 K-Aspartate, 8.5 CaCl\(_2\), 10 K\(_2\)EGTA, 2.0 MgCl\(_2\), and 10 HEPES (pH 7.2), with an estimated free [Ca\(^{2+}\)]\(^{\text{free}}\) of 1 µM (26). KCa3.1 current was measured in voltage-clamp mode by ramp depolarization from −120 mV to +40 mV, 200 ms duration, every 10 s, −80 mV holding potential (HP). Data were corrected for a liquid junction potential of −10 mV (22). The slope conductance of the KCa3.1 current was measured between −100 mV and −60 mV. Kv1.3 currents were induced by depolarization voltage steps from −80 mV HP and applied every 30 s, unless otherwise indicated. The external solution for recording of Kv1.3 currents had the following composition (in millimoles): 150 NaCl, 5 KCl, 2.5 CaCl\(_2\), 1.0 MgCl\(_2\), 10 glucose and 10 HEPES (pH 7.4). The pipette solution was composed of (in millimoles): 134 KCl, 1 CaCl\(_2\), 10 EGTA, 2.0 MgCl\(_2\), 5 ATP-sodium, and 10 HEPES (pH 7.4) (estimated free Ca\(^{2+}\) concentration 10 nM) (27). The number of Kv1.3 and KCa3.1 channels per cell was determined by dividing the channel maximum conductances for their corresponding single channel...
cells lose their peculiar characteristics (abnormalities in Lck, CD45, and lipid rafts) and revert to a normal T cell phenotype (30).
Because the addition of patients’ serum during the in vitro incubation did not prevent this reversion of cell phenotype, it was

Statistical analysis
All data are presented as means ± SEM, unless otherwise indicated. Statistical analyses were performed using Student’s t test (paired or unpaired); p ≤ 0.05 was defined as significant.

Results
Kv1.3 channels in T lymphocytes from patients with SLE display biophysical and pharmacological properties similar to those in healthy T cells
We have performed comparative studies aimed at identifying differences in the expression and activity of K channels in normal T lymphocytes and T lymphocytes from patients with SLE that could explain the enhanced Ca\(^{2+}\) response of the latter cells. The T cell phenotype of the SLE patients enrolled in this study was analyzed by flow cytometry. SLE patients displayed a significant reduction in CD4:CD8 ratio (Fig. 1A) as compared with healthy donors and normal (n = 16) individuals. Normal and SLE T cell populations were further characterized as follows: naive (CCR7\(^{+}\)CD45RO\(^{-}\)), Tcm (CCR7\(^{+}\)CD45RO\(^{-}\)), and Tem (CCR7\(^{-}\)CD45RO\(^{-}\)). There was an increase in CD4\(^{+}\) Tem cells accompanied by a decrease in CD8\(^{+}\) Tem populations in SLE as compared with healthy donors. The levels of significance within the T cell subsets are indicated at the bottom.

FIGURE 1. Expression of T cell subsets in SLE, RA, and normal donors. A, Flow cytometry analysis using anti-CD4 and anti-CD8 Abs as gating Abs of SLE patients (n = 18), healthy controls (n = 16), and RA patients (n = 4). CD4:CD8 ratio indicates the relative proportions of T cells expressing CD4 or CD8. B, Levels of expression of CD4 and CD8 in the three populations. C, Relative levels of naive, Tcm, and Tem cells in CD4\(^{+}\) (left) and CD8\(^{+}\) (right) lineages for SLE (n = 18) and normal (n = 16) individuals. Normal and SLE T cell populations were further characterized as follows: naive (CCR7\(^{+}\)CD45RO\(^{-}\)), Tcm (CCR7\(^{+}\)CD45RO\(^{-}\)), and Tem (CCR7\(^{-}\)CD45RO\(^{-}\)). There was an increase in CD4\(^{+}\) Tem cells accompanied by a decrease in CD8\(^{+}\) Tem populations in SLE as compared with healthy donors. The levels of significance within the T cell subsets are indicated at the bottom.

conductances. The Kv1.3 single channel conductance was determined by us to be 11 pS (28). For KCa3.1, we used the single channel conductance determined by Grissmer et al. (29) and used by others to calculate the number of KCa3.1 channels in T cells in similar experimental conditions (11, 29). Membrane potential was measured by current clamp with the same solutions used to record Kv1.3 currents (22). The cell surface area was determined from the cell capacitance based on the approximation that 1 pF = 100 \(\mu\)m\(^2\) (13). Data were collected using the Axopatch200A amplifier and analyzed with pClamp8 software (Axon Instruments).

Effect of Shk-Dap22 on Kv1.3 current in SLE T cells. Currents were recorded in whole cell configuration in physiologic solution before application of Shk-Dap22 (-Shk-Dap22), after Shk-Dap 22 (10 nM) inhibition and cell configuration and were elicited by depolarizing voltage steps from -60 mV to +40 mV (10 mV increments) from -80 mV HP every 30 s. The conductance-voltage curve (constructed from current amplitudes such as those shown) was fitted to a Boltzman function and the voltage at which half of the channels are activated (\(V_{1/2}\)) calculated. B, Cumulative inactivation of Kv1.3 channels was induced by consecutive depolarizing pulses applied every second. The maximal current amplitude progressively decreased with each successive pulse (indicated by progressive numbers). C, Effect of Shk-Dap22 on Kv1.3 current in SLE T cells. Currents were recorded in whole cell configuration in physiologic solution before application of Shk-Dap22 (-Shk-Dap22), after Shk-Dap 22 (10 nM) inhibition and after drug wash-out (wo). D, Membrane potential (MP) measured by current clamp before and after Shk-Dap22 (10 nM) addition. The time of Shk-Dap22 introduction is indicated by an arrow. The SLEDAI of the patients for this study ranged from 2 to 12.

FIGURE 2. Electrophysiological and pharmacological properties of Kv1.3 channels in SLE T cells. A, Kv1.3 currents were recorded in whole cell configuration and were elicited by depolarizing voltage steps from -60 mV to +40 mV (10 mV increments) from -80 mV HP every 30 s. The conductance-voltage curve (constructed from current amplitudes such as those shown) was fitted to a Boltzman function and the voltage at which half of the channels are activated (\(V_{1/2}\)) calculated. B, Cumulative inactivation of Kv1.3 channels was induced by consecutive depolarizing pulses applied every second. The maximal current amplitude progressively decreased with each successive pulse (indicated by progressive numbers). C, Effect of Shk-Dap22 on Kv1.3 current in SLE T cells. Currents were recorded in whole cell configuration in physiologic solution before application of Shk-Dap22 (-Shk-Dap22), after Shk-Dap 22 (10 nM) inhibition and after drug wash-out (wo). D, Membrane potential (MP) measured by current clamp before and after Shk-Dap22 (10 nM) addition. The time of Shk-Dap22 introduction is indicated by an arrow. The SLEDAI of the patients for this study ranged from 2 to 12.
suggested that interaction with other cell types might be responsible for the alteration in proximal signaling pathways (30). We found that Kv1.3 currents in SLE T cells were voltage dependent with a $V_{1/2}$ (voltage at which half of the channels are activated) of $−27 ± 1$ mV ($n = 18$) similar to that of normal resting T cells ($−25 ± 1$ mV, $n = 6, p = 0.27$) (12, 33) (Fig. 2A). The activation and inactivation time constants of Kv1.3 currents measured at $+50$ mV in SLE T cells were also similar to those of Kv1.3 current in healthy resting T cells. The activation time constants were 163 ± 13 ms ($n = 52$) in healthy T cells and 186 ± 29 ms ($n = 20$; $p = 0.5$) in SLE T cells. The time constants of inactivation in healthy and SLE resting T cells were 207 ± 19 ms ($n = 52$) and 247 ± 16 ms ($n = 20, p = 0.1$), respectively. Furthermore, Kv1.3 channels in SLE T cells display cumulative inactivation, a characteristic property of these channels, as indicated by the progressive decrease of the maximal current amplitude upon application of consecutive depolarizing pulses every second (Fig. 2B) (8). Additional studies established that the Kv1.3 currents recorded in SLE T cells were completely and reversibly blocked by the selective Kv1.3 inhibitor ShK-Dap22 (10 nM, Fig. 2C) and this concentration of

![Image](http://www.jimmunol.org/)

**FIGURE 3.** Kv1.3 channels are recruited at the interface between CD3/CD28 beads and T cells. A–C, Normal (A), SLE (B), and RA (C) human CD3+ cells were stimulated with CD3/CD28 beads for 5–15 min at 37°C. Resting (nonexposed to beads, top panels) or bead activated T cells (bottom panels) were fixed, permeabilized, immunolabeled for Kv1.3 (green) and either F-actin (A, left panel, B and C) or GM1 (A, right panel) and visualized by confocal microscopy. F-actin and GM1 were identified by fluorescence-labeled phalloidin and fluorescence-labeled cholera toxin B, respectively (red). Areas of colocalization of Kv1.3 channels and F-actin or GM1 are shown in yellow in the right panels (merge). D, T cells were exposed to either CD3/CD28 or CD19 beads for 15 min at 37°C. Resting (nonexposed to beads, top left panel), CD19 exposed T cells (top middle and right panels) or CD3/CD28-activated T cells (bottom panels) were fixed, permeabilized, immunolabeled for Kv1.3 (green) and F-actin (red) and visualized with fluorescence microscopy. Beads are marked with an X. Scale bar, 5 μm. E, Distribution of the MFR in T/bead conjugates that form in presence of either CD19 or CD3/CD28-coated beads.

**Table I.** $K$ channel expression in SLE and normal T lymphocytes

<table>
<thead>
<tr>
<th>$K$ Channel</th>
<th>Normal</th>
<th>Activated</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.3 Capacitance (pF)</td>
<td>1.01 ± 0.04</td>
<td>4.46 ± 0.19***</td>
<td>1.49 ± 0.08***,†††</td>
</tr>
<tr>
<td>$n$</td>
<td>62</td>
<td>55</td>
<td>40</td>
</tr>
<tr>
<td>Current density (pA/pF)</td>
<td>501 ± 36</td>
<td>263 ± 27***</td>
<td>416 ± 40†</td>
</tr>
<tr>
<td>Total channels (no. channels/cell)</td>
<td>308 ± 16</td>
<td>786 ± 83***</td>
<td>349 ± 30†††</td>
</tr>
<tr>
<td>Channel density (no. channels/μm$^2$)</td>
<td>3.50 ± 0.25</td>
<td>1.84 ± 0.19***</td>
<td>2.91 ± 0.28*</td>
</tr>
<tr>
<td>$n$</td>
<td>52</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>KCa3.1 Conductance (nS)</td>
<td>0.33 ± 0.03</td>
<td>2.55 ± 0.27***</td>
<td>0.34 ± 0.03†††</td>
</tr>
<tr>
<td>Total channels (no. channels/cell)</td>
<td>29.7 ± 3.3</td>
<td>232.0 ± 24.9***</td>
<td>30.7 ± 2.5†††</td>
</tr>
<tr>
<td>Channel density (no. channels/μm$^2$)</td>
<td>0.24 ± 0.03</td>
<td>0.57 ± 0.07**</td>
<td>0.21 ± 0.02†††</td>
</tr>
<tr>
<td>$n$</td>
<td>10</td>
<td>32</td>
<td>20</td>
</tr>
</tbody>
</table>

*a***, $p < 0.01$ vs resting; ***, $p < 0.0001$ vs resting; †, $p < 0.005$ vs activated; †††, $p < 0.0001$ vs activated.
ShK-Dap22 induced a 23 ± 4 mV (n = 4, Fig. 2D) membrane depolarization in these cells (34). We have also estimated whether T lymphocytes from SLE patients expressed the same number of Kv1.3 channels as healthy resting T cells. The total number of Kv1.3 channels/cell was determined by dividing the channel maximum conductance for its corresponding single channel conductance (11 pS) (28). SLE T cell express on average 349 ± 30 channels/cell (n = 20), ranging between 178 and 675 channels/cell. This is similar to the number of channels expressed by normal resting T cells (308 ± 16, n = 52; p = 0.2) (Table I).

Taken together, these data demonstrate that SLE T cells expressed the same number of Kv1.3 channels as resting T cells from healthy donors and that these channels share identical biophysical and pharmacological properties with their healthy counterparts. Moreover, Kv1.3 channels control the membrane potential in SLE T cells as indicated by the depolarization induced by Kv1.3 channel blockade.

Native Kv1.3 channels are recruited in the immunological synapse upon activation of healthy and SLE T cells

Because the biophysical properties of the channel remained unaltered, we wanted to investigate whether other alterations in the Kv1.3 channel behavior might be encountered in SLE T cells. Previous studies have shown that recombinant Kv1.3 channels are recruited in the IS (14–16). However, the process by which native Kv1.3 channels transition into the IS is still to be defined. Furthermore, possible alterations of this process in diseased T cells have never been investigated. To address this question, we first investigated Kv1.3 channel polarization to the synapse in human CD3 T cells from healthy donors. To induce T cell activation, and synapse formation, we used anti-CD3/CD28 Ab-coated beads as surrogate APCs. This is a well-validated system to study membrane reorganization and downstream functional events triggered by TCR binding (22, 23). Our results indicate that upon stimulation with CD3/CD28 beads, Kv1.3 channels partition to the T cell/bead contact area and colocalize extensively with F-actin and the glycosphingolipid GM1, a marker of lipid rafts (Fig. 3, bottom panels). Both F-actin and GM1 are known to reorganize and accumulate at the IS (18, 35). In contrast, Kv1.3 channels are evenly distributed on the membrane of resting T cells not exposed to beads (Fig. 3, top panels). In the same way, SLE and RA T cells recruit Kv1.3 channels in the cell/bead contact interface upon activation with the CD3/CD28 beads (Fig. 3, B and C, lower panels) while the channels remain evenly distributed in the absence of beads (Fig. 3, B and C, upper panels). To exclude that Kv1.3 channel relocation occurs because of simple cell-to-bead
Kv1.3 channel compartmentalization in the immunological synapse is altered in SLE T cells

We studied the process of Kv1.3 channel translocation in the IS in SLE, RA, and normal donors. Fourteen SLE patients were included in the following microscopy studies: 11 females and 3 males, 10 AA and 4 C, age, 38.0 ± 3.1 years (p = 0.3 vs healthy individuals), range 24–67 years. These patients’ SLEDAI ranged from 2 to 12. As controls, we used nine normal subjects: 5 females, 2 males, and 2 unknown, 2 AA, 5 C, and 2 unknown, age 43.3 ± 3.1 years; range 33–53 years and 5 RA patients: 5 female, 3 AA, and 2 C, age 57 ± 6.0 and 1 unknown years, range 40–68.

We first examined the kinetics of Kv1.3 channel recruitment into the IS in resting T cells from healthy individuals by exposing them to CD3/CD28 beads for 1, 5, 15, and 30 min. Cell conjugates formed between CD3/CD28 beads and T cells were then fixed and immunostained with anti-Kv1.3 Ab. The assessment of the time-dependent distribution of Kv1.3 channels in the IS was done by establishing the number of T cell/bead conjugates with polarized Kv1.3 proteins over the total number of conjugates for each time point. Fig. 4A indicates that Kv1.3 channel redistribution in the IS of resting healthy T cells occurs after only 1 min of exposure to the beads and progressively increases over time. Overall Kv1.3 recruitment in the IS is maintained for at least 30 min from synapse formation. Still at 1 h, there was sustained recruitment. The percentage of Kv1.3 polarized conjugates at 1 h was 53 ± 5% (n = 2) (data not shown). By 2–5 h the Kv1.3 channel was removed from the synapse with only 25 ± 4% conjugates showing Kv1.3 recruitment (n = 2) (data not shown). Similar experiments were performed with SLE T cells and we observed that in seven of eight patients, the kinetics of Kv1.3 channel compartmentalization in the synapse are quite different (Fig. 4B). Specifically, Kv1.3 polarization in primary SLE T cells is maximal at 1 min after TCR engagement and progressively declines over time, indicating that Kv1.3 channels either redistribute on the plasma membrane outside the IS or that they are internalized and degraded. This defect appears to be restricted to SLE as it was not observed in RA patients (Fig. 4C). Although some degree of variability was observed in individual RA patients, we never encountered contact, to establish the variability of our technique, and to determine the threshold for a significant Kv1.3 channel accumulation in the synapse, we performed identical experiments using beads coated with an Ab against CD19 (a component of the BCR complex) (23). In contrast to CD3/CD28 beads, CD19-coated beads did not have a significant effect on Kv1.3 or F-actin localization to the cell/bead contact interface (Fig. 3D).

FIGURE 5. APC-T cell activation induces differential reorganization of Kv1.3 channels in the IS formed with resting healthy and SLE T cells. A. Comparable effectiveness of anti-Kv1.3 Abs against an IC epitope (IC Ab) and an EC epitope (EC Ab) in detecting Kv1.3 channel accumulation in the IS. Resting T cells and T cells activated with CD3/CD28 beads are shown for each Ab (left and right images, respectively). T cells were either mixed with the beads and immunolabeled, after fixation, with anti-Kv1.3 IC Ab or labeled live with the anti-Kv1.3 EC before interaction with the beads. CD3/CD28 beads are marked by an X. B. Accumulation of Kv1.3 and CD3ε in the IS. Healthy resting T cells were incubated with EBV-infected B cells that had been exposed to medium with (bottom panels) or without (top panels) SEB. B cells were labeled with CMAC cell tracker blue. C. Accumulation of Kv1.3 and CD3ε in the IS formed between APCs and SLE T cells after 1 and 30 min interaction. Scale bar, 5 μm. D and E, Time-dependent recruitment of Kv1.3 channels in the IS in healthy and SLE T cells. T/B cell conjugates were quantitatively evaluated for the recruitment of Kv1.3 channels as described in Materials and Methods. The data are reported as average of the relative percentage of Kv1.3-polarized conjugates (normalized for the maximum recruitment of Kv1.3-polarized conjugates). The histograms represent 3 healthy donors (3 C) and 3 SLE patients (1 AA and 2 C, SLEDAI range: 2–8). At least 10 T/APC conjugates were evaluated for each donor per time point.

Kv1.3 kinetics matching SLE T cells and on average, Kv1.3 channels are recruited in the IS of RA T cells within 1 min and are maintained there for at least 30 min.
This differential kinetics of Kv1.3 translocation into the IS in healthy and SLE T cells were also observed to occur at the interface between T cells and APCs (Fig. 5). T cells were incubated with EBV-infected B cells in the presence or absence of SEB for 1 and 30 min and the accumulation of Kv1.3 and CD3e at the IS was determined. Control experiments were performed using EBV-infected B cells in the absence of SEB. To study the compartmentalization of Kv1.3 channels in the contact area between T cells and SEB-pulsed EBV-infected B cells, we labeled the T cells “live” with an anti-Kv1.3 Ab against an EC epitope of the Kv1.3 channel protein before encounter with the APCs. This allowed selective labeling of the Kv1.3 channels in the T cell membrane and not those expressed in the B cells (36). This Ab is specific for Kv1.3 channels as determined by the lack of fluorescence signal after preadsorption of the Ab to the corresponding Ag (data not shown) and it can be used alternatively to the Ab for an EC epitope of the Kv1.3 channel that we have used in T cell/bead experiments. Similar results were obtained using the two Abs (Fig. 5A). The accumulation of Kv1.3 channels in the IS was determined as described in Materials and Methods and B/T cell conjugates that displayed a fluorescence at the synapse ≥50% of the total fluorescence were defined as polarized Kv1.3 conjugates. Our results indicate that in the absence of SEB, Kv1.3, and CD3e were evenly distributed on the plasma membrane of healthy T cells in the majority of the conjugates while in the presence of SEB Kv1.3 and CD3e concentrated at the IS (Fig. 5B). Overall, normal resting T cells showed Kv1.3 polarization to the IS at 1 min and the channels were maintained in the IS for at least 30 min (Fig. 5, B and D). All the cells that recruited Kv1.3 also recruited CD3e. A different pattern of translocation into the IS that forms with APCs was instead observed with SLE T cells (Fig. 5, C and E). Kv1.3 polarization in SLE T cells is maximal at 1 min after TCR engagement and is decreased by 30 min. These results substantiate, in a more physiological model of T/APC interaction, the observations made with the CD3/CD28-coated beads.

The kinetics of Kv1.3 redistribution in the immunological synapse of SLE T cells resemble those of preactivated normal T cells

It is generally believed that SLE T cells exist in an active state (37). Accordingly, it is possible that the different dynamics of Kv1.3 compartmentalization in SLE T cells might reflect a more activated T cell phenotype. We have thus studied the process of Kv1.3 compartmentalization upon TCR engagement in PHA preactivated healthy T cells (Fig. 6). Although resting T cells display a long-lasting recruitment of Kv1.3 channels in the IS (Figs. 4 and 5), preactivated T cells display a different time course: Kv1.3 channels moved rapidly to the IS with maximal recruitment at 1 min and progressively moved out of the synapse by 30 min (Fig. 6). Instead, in the absence of stimulation, Kv1.3 channels remained evenly distributed around the membrane. Consistent results were obtained using either CD3/CD28 beads or SEB-pulsed B cells as APCs. Overall, the dynamics of Kv1.3 compartmentalization in healthy activated T cells parallel the Kv1.3 recruitment observed in resting SLE T cells.

The process of Kv1.3 channel translocation in the IS during activation with CD3/CD28 beads was quantitatively summarized by determining the rate of change in number of Kv1.3-polarized conjugates over time using a linear regression model. The “slope” of the model, indicative of the rate of formation of Kv1.3-polarized conjugates, was compared in SLE patients and normal controls (Fig. 7A). Overall, we observed similar negative slopes in seven of eight SLE patients, indicating that in these patients the localization of Kv1.3 in the IS is short-lived. Similar slopes were observed in activated healthy T cells and they were significantly different from those determined in healthy resting T cells. This behavior appeared to be unrelated to the disease activity and immunosuppressive regime. When we grouped all the SLE patients that were used in the microscopy studies, using either CD3/CD28-coated beads or APCs, we observed this Kv1.3 mobility defect in all except one patient. The outlier (SLE patient 8, Table II) displayed kinetics similar to normal resting T cells. This patient also showed a particular immunosuppressed T cell phenotype as indicated by a percentage of naive CD4+ and CD8+ cells well above healthy controls (70 and 84%, respectively). This raised the possibility that the abnormal Kv1.3 behavior in SLE T cells might be determined by the more activated state of their CD4 lineage (Fig. 1). If this is the case, we would expect that CD4+ and not CD8+, display these characteristic kinetics. Experiments were thus performed to compare the rate of Kv1.3-polarized conjugate formation in CD4+ and CD8+ cells from four SLE patients. We observed, on average, no differences between these T cell subsets (Fig. 7B).

Overall, these results establish that the general SLE T cell population displays faster kinetics of Kv1.3 channel translocation in

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**FIGURE 6.** Kv1.3 channel recruitment in the IS in activated healthy T cells parallels SLE T lymphocytes. A. T cells were preactivated by exposure to PHA (4 μg/ml) in the presence of autologous PBMCs for 72 h. Preactivated T cells were or were not stimulated with CD3/CD28 beads for 1–30 min. Left panels, Representative photomicrographs. Right panels, Quantitative analysis of Kv1.3 channel recruitment in the IS of activated T cells was performed as described in Materials and Methods. The histogram shows the percentage of cells showing Kv1.3 accumulation at the IS at different time points (1–30 min). The data are the average of >50 cells/donor from seven healthy donors except 5 min, six donors. B. Activated healthy T cells were incubated with SEB-pulsed EBV B cells for 1 or 30 min. T/B cell conjugates were quantitatively evaluated for the recruitment of Kv1.3 channels as described in Materials and Methods. The histograms represent an average of the relative percentage of Kv1.3-polarized conjugates (normalized for the maximum recruitment of Kv1.3-polarized conjugates) in three healthy donors. At least 10 T/APC conjugates were evaluated for each donor per time point.
It has been shown that Kv1.3 channels, and not Kv1.3 channels, control Ca$$^{2+}$$ homeostasis in activated cells (26). So it is possible that the rapid dynamics of the Kv1.3 translocation into the IS in preactivated T cells are compensated by the presence of KCa3.1 channels. The expression of KCa3.1 channels in SLE T cells has yet to be determined. Experiments were thus performed to determine whether the K channel expression in SLE T cells matches preactivated healthy T cells.

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Whole cell voltage-clamp experiments were performed to determine the expression of KCa3.1 channels in SLE T cells and compare it to that of healthy T cells (Fig. 8 and Table I). The healthy T cells studied consisted of both resting (freshly isolated) and mitogen preactivated T cells obtained by prolonged exposure to PHA. This intervention has been shown to activate human T cells and increase their cell capacitance, a measure of cell size, and KCa3.1 conductance (26, 38). These cells also showed a faster Kv1.3 channel compartmentalization upon TCR activation (Fig. 6). Membrane capacitance measurements indicated that the activated T cells we studied were indeed activated. The membrane capacitances of mitogen preactivated and resting (freshly isolated) CD3$$^+$$ cells were 4.46 ± 0.19 pF (n = 55) and 1.01 ± 0.04 pF (n = 62; p < 0.001), respectively (Table I). Similar capacitance values have been reported for quiescent and preactivated human T cells (39). Interestingly, we found that resting SLE T cells have membrane capacitance higher than resting healthy T cells, but less than preactivated T cells. This indicates that resting SLE T cells are bigger than resting healthy T cells with an average cell surface area of 149 and 110 μm$$^2$$, respectively (Fig. 8B). The cell surface area was determined from the cell capacitance based on the approximation that 1 pF = 100 μm$$^2$$. This might indicate that SLE T cells are partially activated or are “frozen” in an early stage of activation as previously suggested (37). Yet, the KCa3.1 conductance in SLE T cells is identical to normal resting T cells, suggesting that the number of channels is the same (Table I). Indeed, the KCa3.1 channel number/cell in SLE T cells is similar to that of primary resting T cells (Fig. 8C and Table I). In contrast, healthy preactivated T cells have an 8-fold increase in KCa3.1 conductance which translates to an 8-fold increase in channel numbers. When normalized for cell size, SLE T cells have KCa3.1 channel density similar to resting T cells and significantly lower than healthy preactivated T cells (Table I). Similarly, the Kv1.3 channel density in SLE T cells is comparable to healthy primary T cells. The Kv1.3 and KCa3.1 channel composition in the mixed population of normal (resting and activated) and SLE T cells is summarized in Table I. These results indicate that the number of functional Kv1.3 and KCa3.1 channels

TABLE II. Details of SLE and RA patients in the microscopy studies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>SLEDAI</th>
<th>Medications $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>HCQ + MMF + Pred.*</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>Azathioprine + Pred.*</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>HCQ + Pred.*</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Pred.</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>HCQ + Pred.*</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
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</tr>
<tr>
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<td>2</td>
<td>HCQ + MMF</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>HCQ + Pred. + MMF</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>Pred. + MMF</td>
</tr>
<tr>
<td>10</td>
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<td>12</td>
<td>4</td>
<td>Pred.*</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>HCQ + Pred.</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>HCQ + Pred. + Azathioprine</td>
</tr>
</tbody>
</table>

RA patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pred.</td>
</tr>
<tr>
<td>2</td>
<td>HCQ + Pred.*</td>
</tr>
<tr>
<td>3</td>
<td>HCQ + MTX.</td>
</tr>
<tr>
<td>4</td>
<td>HCQ</td>
</tr>
<tr>
<td>5</td>
<td>HCQ + MTX + Pred.*</td>
</tr>
</tbody>
</table>

$^a$ HCQ, hydroxychloroquine (Plaquenil); MMF, mycophenolate mofetil (CellCept); MTX, methotrexate; Pred., prednisone; *, ≤10 mg.

FIGURE 7. Comparison of the rates of Kv1.3 channel compartmentalization in the IS in normal and SLE T cells. A, The rate of Kv1.3-polarized conjugate formation induced by activation with CD3/CD28 beads was determined in normal and SLE T lymphocytes by linear fitting of the time courses shown in Figs. 4, C and D, and 6B. The slope of the model is plotted for each group: normal lymphocytes (NL), resting (○), and SLE (●) T cells. A negative slope is indicative of rapid Kv1.3 channel redistribution outside the IS. B, Rate of Kv1.3-polarized conjugate formation in CD4$$^+$$ and CD8$$^+$$ cells from SLE patients. CD4$$^+$$ and CD8$$^+$$ cells were separated from the same individual and studied in parallel. A total of 4 SLE patients were studied: 3AA and 1C. SLEDAI range 4–10. T cells were activated by exposure to CD3/CD28 beads for 1 and 30 min. The number of Kv1.3-polarized conjugates for each time point was determined as described in the legend of Fig. 4 and plotted against time. The slope obtained by linear fitting of the time course is reported. The symbols indicating each donor are conserved among the two groups.
expressed in SLE T cells is similar to that of healthy resting T cells. Thus, Kv1.3 channels constitute the main K conductance in SLE T cells and as such are the main regulators of membrane potential and Ca$^{2+}$ homeostasis in these cells.

**Discussion**

In this study, we examine for the first time K channels in SLE T cells and provide evidence of significant differences in Kv1.3 channel dynamics of translocation in the IS between resting healthy and SLE T lymphocytes. We also find that in SLE T cells, the movement of the Kv1.3 channel on the plasma membrane during presentation of the Ag and formation of the IS resembles that of healthy activated T cells. Despite that, SLE T cells lack the corresponding K channel make-up that is integral in regulating the activation response in normal activated T cells. These discrepancies might account for the hyperactivity and exaggerated response of SLE T cells to Ag presentation.

K channels have been shown to be key regulators of T cell activation as they control the membrane potential and the influx of Ca$^{2+}$ triggered by Ag presentation (8). As such, K channels could play a role in the abnormal Ca$^{2+}$ response to Ag stimulation that has been reported to occur in human SLE T lymphocytes (5, 6). Nevertheless, their activity and function in SLE T cells has never been investigated. We have conducted various studies aimed at dissecting the properties of K channels in SLE T cells. These studies were conducted on a cohort of SLE patients with a T cell phenotype characteristic of this disease as indicated by the low CD4−CD8 ratio (30, 31). This low CD4−CD8 ratio might have been exacerbated by the presence of patients with lupus nephritis and patients under corticosteroid treatment (40). Furthermore, the SLE patients display an activated CD4$^+$ memory phenotype with CD4$^+$ Tem levels higher than healthy individuals as previously described (41). This is accompanied by a decreased expression of CD8$^+$ Tem cells and it is in agreement with the common consent that the altered immune response in SLE is mediated by an imbalance in the functions of T cell subsets: exaggerated activity of CD4$^+$ helper cells and diminished function of CD8$^+$ suppressor/cytotoxic cells (3).

When we analyzed this mixed population of SLE T cells for the expression of K channels, we found that SLE T cells display a K channel phenotype similar to normal resting T cells with Kv1.3 channels constituting the main K conductance and controlling the membrane potential. Although we showed no differences in the biophysical and pharmacological properties of Kv1.3 channels in SLE T cells as well as in their number as compared with normal resting T cells, our data indicate that there are fundamental differences in the process of Kv1.3 channel translocation in the IS. The accumulation of Kv1.3 channels in the IS in healthy resting T cells occurs progressively and it is sustained for a long time, well beyond the onset of signal transduction (i.e., the onset of Ca$^{2+}$ influx) (42). This is consistent with the long time necessary for resting T cells to form mature synapses (43). In contrast, SLE T cells show a faster recruitment of Kv1.3 channels into the IS and redistribution outside the synapse. Interestingly, the process of Kv1.3 recruitment in SLE T cells parallels the process observed in healthy preactivated T cells. Indeed, it has been shown that synapse maturation occurred much faster in T cell blasts than resting T cells (43). This behavior of SLE T cells is consistent with the view that T cells from SLE patients resemble activated T cells (37). T cells from patients with SLE display various characteristics of activated T cells: they exhibit a loss of CD3ζ chain which is replaced by FcRγ chain and Syk recruitment to the TCR complex. These alterations indicate a switch from the TCR/CD3/CD3ζ/Zap70 receptor complex of resting T cells to the TCR/CD3/FcRγ/Syk receptor complex of effector T cells (37). Functionally, SLE T cells are primed for activation and respond more rapidly to antigenic triggers than do T cells from normal individuals (30). Furthermore, they react more rapidly than healthy T cells to Ag presentation in terms of reorganization of elements known to accumulate at the IS such as F-actin and lipid rafts (6). Along this line, the significantly larger size of SLE T cells we have measured during our electrophysiological experiments indicates that, in these patients, T cells exist in a partially activated state as previously suggested (37). Similar to our findings, all these alterations were found in freshly isolated peripheral blood T cells from SLE.
patients independent of their disease activity, thus suggesting a constant activation state of SLE T cells (6, 31).

However, although SLE T cells circulate as activated (or partially activated) T cells, they do not display the K channel make-up characteristic of activated T cells. SLE T cells express ~300 Kv1.3 and ~30 KCa3.1 channels/cell. Similar values are reported in the literature for resting naive, Tcm and Tem cells of the CD4 and CD8 lineages (13). Although we were measuring a mixed population, we never encountered SLE T cells with either high Kv1.3 channel number (≥1500/cell), indicative of the activated Tem phenotype, or high KCa3.1 channel number, indicative of activated naive and Tcm cells (13). Overall, freshly isolated peripheral blood SLE T cells express a number of Kv1.3 and KCa3.1 channels equal to resting healthy T cells and, likewise, Kv1.3 channels constitute the main K conductance in SLE T cells. As such, they modulate SLE T cell membrane potential. Thus, alterations in Kv1.3 channel behavior might have important consequences in the Ca\(^{2+}\) homeostasis of SLE T cells. It is possible that alterations in dynamics of Kv1.3 localization in the IS contribute to the pronounced and sustained TCR-mediated Ca\(^{2+}\) influx of SLE T cells. This exaggerated Ca\(^{2+}\) response was observed in both SLE CD4\(^+\) and CD8\(^+\) subsets, although it was higher in CD4\(^+\) (5). Consistently, we showed that both CD4\(^+\) and CD8\(^+\) SLE T cells display faster dynamics of Kv1.3 translocation in and out of the IS. Furthermore, we showed that this defect is not present in T cells from RA patients. This is consistent with the fact that RA T cells do not display an exaggerated Ca\(^{2+}\) response to Ag presentation (5, 44).

The functional consequences of this differential dynamics of Kv1.3 protein localization in the IS of the SLE T cells are unclear at present. However, it has been suggested that ion channel localization in the IS might be necessary for guaranteeing the channel proximity to signaling molecules that control the channel’s function (45). The data we have presented indicating that in healthy resting T cells the Kv1.3 channels are maintained in the IS for ~2 h are consistent with the notion that a prolonged interaction of naive T cell with APC lasting 2 h or more is required for cell division and IL-2 production/release from the cell (43, 46, 47). Although it has been shown that tyrosine phosphorylation activation mechanisms and the initial Ca\(^{2+}\) influx occur early upon T cell contact with the Ag (within 2–15 min), other signaling systems such as those involving Ca\(^{2+}\) or serine/threonine phosphorylation have been suggested to be critical during the later stages of activation. Because Kv1.3 channels are known regulators of Ca\(^{2+}\) homeostasis in human T cells and their activity is modulated by serine/threonine kinases, it is very likely that they constitute key components of the late activation signaling complex. The prolonged time Kv1.3 channels reside in the IS may indeed be necessary for the channels to come in close proximity with signaling molecules also recruited at the IS thus facilitating the regulation of their activity and consequently the control and termination of the Ca\(^{2+}\) response. It has been shown that various elements that accumulate at the IS such as cholesterol and lipid rafts as well as Lck, PKC, and protein kinase A can modulate Kv1.3 channel function (28, 48–53). Furthermore these kinases move into the IS at different times after Ag presentation, with protein kinase A and PKC\(\theta\) still present at the IS well after a mature synapse is formed (54–56). Our results suggest a model in which a proper time-dependent localization of Kv1.3 in the IS is necessary for its regulation. In normal resting T cells, the Kv1.3 channel remains in the IS for the time necessary for its regulation. This process is responsible for bringing Kv1.3 channels into close physical proximity with signaling molecules would have particular biological relevance in the setting of SLE where there is a documented decrease in the expression and activity of multiple kinases (3). Unfortunately, because Kv1.3 channels in SLE T cells leave the IS prematurely they might not be properly regulated and an abnormal Ca\(^{2+}\) response might develop. In contrast, a prolonged localization of Kv1.3 channels is instead not necessary in normal activated T cells because they also express high levels of KCa3.1 channels that could control Ca\(^{2+}\) homeostasis (26). We have recently shown that KCa3.1 channels are recruited in the IS of activated T cells where they reside for 30 min (17).

The data presented herein raise the possibility that Kv1.3 channels might be involved in the pathophysiology of SLE. Given the availability of pharmacological agents altering these channels, these data may lead to the discovery of new therapeutic targets for this disease.

Acknowledgments

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Disclosures

The authors have no financial conflict of interest.

References


