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Molecular mechanisms for Kv1.3 potassium channel current inhibition by CD3/CD28 stimulation in Jurkat T cells

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ABSTRACT

In T lymphocyte, activation of Kv1.3 channel, the major voltage-dependent K^+ channel, is an essential step for cell proliferation in immune responses. Here, effects of anti-CD3 and anti-CD28 antibodies on Kv1.3 current were examined in three types of human T lymphocyte derived cell lines, Jurkat E6-1, p56lck-kinase deficient mutant JCaM.1, and CD45-phosphatase deficient mutant J45.01. Kv1.3 current was partly reduced by CD3 stimulation and more strongly by addition of anti-CD28 antibody in E6-1. In JCaM.1, Kv1.3 current responses to anti-CD28/CD3 antibodies were similar to those in E6-1. In J45.01, CD3 stimulation partly inhibited Kv1.3 current, but the additive reduction by CD28 stimulation was not significant. The inhibition of tyrosine phosphatase in E6-1 abolished the additional inhibition by anti-CD28 antibody in a similar manner as in J45.01. In conclusion, the stimulation of CD28 in addition to CD3 strongly inhibits Kv1.3 current and this additive inhibition is mediated by CD45 activation.

In the regulation of immune responses, co-stimulation of two signals is required for the full activation of T lymphocytes. The first signal is mediated by cross-linking of the T cell receptor (TCR). The second signal is mediated by co-stimulatory molecules that interact in antigen presenting cells. It has been reported that the CD3/CD28 co-stimulation leads to higher tyrosine phosphorylation of several substrates, autophosphorylation of p56lck and activation of phospholipase Cγ, which facilitates inositol 1,4,5-trisphosphate (IP_3) formation and induces Ca^{2+} release from intracellular store [1,2]. The depletion of Ca^{2+} store causes opening of Ca^{2+} released-activated Ca^{2+} (CRAC) channel and subsequent Ca^{2+} flow into the cells [3,4]. This sustained influx of Ca^{2+} causes both the interleukin-2 production and the T cell proliferation [5].

Activities of ion channels regulate the membrane potential in excitable and non-excitable cells. In non-excitable cells, when potassium channels are activated, cells are hyperpolarized and the intracellular sustained Ca^{2+} signal is increased through Ca^{2+}-permeable channels such as CRAC channels. In T lymphocyte, two major potassium channels, the voltage-gated Kv1.3 and Ca^{2+}-activated potassium (K_{Ca}) channels are expressed [6]. These channel expression depends on the T cell activation state. In resting naive and central memory T cells as well as resting and activated effecter memory T cells, Kv1.3 channels predominantly contribute to K^+ conductance and regulate Ca^{2+} influx via membrane potential changes [6]. The expression of K_{Ca} is up-regulated, when native and central memory T cells are activated [6].

In earlier works, it has been shown that the blockade of these potassium channels in T lymphocytes depolarizes cell membrane and inhibits cell proliferation [7,8]. The treatment with a selective Kv1.3 channel blocker ameliorated the autoimmune animal disease models such as arthritis, type I diabetes mellitus and multiple sclerosis [4,9,10]. The human leukemic T cell line Jurkat also expresses voltage-dependent Kv1.3 channel and K_{Ca} channel [11,12] and is often used as a model system in T cell activation researches in this line of studies.

Kv1.3 channels are tyrosine phosphorylated by src kinase [13]. The src kinase inhibition by herbimycin A or the deficiency of the p56lck tyrosine kinase in JCaM1 cells abolished Kv1.3 channel phosphorylation and the inhibition of channel activity by stimulation with anti-Fas antibody [12]. Acute effects of CD3 stimulation and CD3/CD28 co-stimulation on Kv1.3 current are, however, undetermined yet to our knowledge. In this study, using Jurkat and mutant Jurkat cells, we examined Kv1.3 current regulation by anti-CD3/CD28 antibody stimulation and revealed the current reduction and underlying molecular mechanisms.

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Methods and materials

Cell culture. The human leukemia T cell line, Jurkat E6-1, p56lck-deficient Jurkat mutant, JCAM.1, and CD45 deficient Jurkat mutant, J45.01 were obtained from ATCC. These Jurkat cell lines were grown in RPMI1640 medium containing 10% heat inactivated FBS, 10 mM Hepes, and 2 mM glutamate. Cells were cultured in culture plate at 37 °C in 5% CO_2 humidified incubator.

Electrophysiological measurements. Membrane currents were measured in Jurkat cells using standard whole-cell voltage-clamping, using CEZ-2300 Amplifier (Nihon-Koden, Japan) and signals were stored on videotape using a PCM system and were later captured on an IBM computer using DT2801A as an analog–digital converter and an acquisition program for precise analysis as ahs been reported previously [14]. All experiments were carried out at 23 ± 2 °C.

Solutions. The physiological salt solution for electrophysiological experiments contained mM: NaCl 137, KCl 5.9, $MgCl_2$ 1.2, glucose 10, Hepes 10. The pH was adjusted to 7.4 with NaOH. The pipette solution for electrical recordings contained mM: KCl 30, KAspartate 110, $ATPNa_2$ 2, $MgCl_2$ 2, EGTA 0.05, Hepes 10. The pH was adjusted to 7.2 with KOH.

Chemicals. Purified mouse anti-human CD3 (clone OKT3) was purchased from eBioscience. Purified mouse anti-human CD28 (clone CD28.2) was purchased from BD Pharmingen. Both antibodies were diluted with distilled water before use. Dipotassium oxodiperoxo (pyridine-2-carboxylato) vanadate (bV(pic)) was purchased from Calbiochem, and was solved with distilled water to 1 mM stock solution and stocked at −20 °C prior to use. Margatoxin was purchased from Peptide Institute Inc. and solved with distilled water to 0.1 mM stock solution and stocked at −20 °C prior to use.

Statistical analysis. Data are expressed as means ± SEM in the text. Statistical significance between two or among multiple groups was examined using Student's *t* or Tukey's test after *F*-test or one way ANOVA, respectively. Significance was expressed in figures by $^{*}p < 0.05$ or $^{**}p < 0.01$.

Results

Effects of anti-CD3 and anti-CD28 antibodies on Kv1.3 current in Jurkat cells

In Jurkat T lymphocyte E6-1, outward currents were elicited by depolarization to +40 mV under whole-cell voltage-clamp. Kinetics of outward currents show rapid activation and slow inactivation and were typical for voltage-gated Kv1.3 channels (Fig. 1A). The current density at the peak was 31.7 ± 11.5 pA/pF ($n = 9$). Application of 10 nM margatoxin, a selective blocker of Kv1.3 channels [15], reduced the outward current by over 90%, confirming that Kv1.3 is the predominant component of the outward current. In this study, MgTX was added at end of each experiment to evaluate Kv1.3. The Kv1.3 currents was stably recorded for over 20 min, when depolarization was applied every 15 s. Stimulation of E6-1 with 2 or 10 μg/ml anti-human CD3 antibody slowly but significantly reduced Kv1.3 current to 85.3 ± 10.6% (relative to before administration, $n = 4$), and to 74.1 ± 4.6% ($n = 7$), respectively (Fig.1A and B). Application of anti-CD28 antibody alone did not affect Kv1.3 current (data not shown). The addition of 10 μg/ml anti-CD28 antibody in the presence of anti-CD3 antibody induced further decrease in Kv1.3 current from 76.2 ± 5.8% ($n = 5$) to 53.4 ± 7.2% ($n = 5$, $p < 0.05$) (Fig. 1D and E).

Simultaneous application of 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 antibodies exhibited slowly developing but marked inhibition of Kv1.3 current. After 20 min from the start of the application, the current reduced to 44.0 ± 0.56% ($n = 5$) (Fig. 2A and B). Fig. 2C shows that the current–voltage relationships before and after the application of antibodies and after the block by MgTX. It is notable that the low concentration (1 μg/ml) of anti-CD3/anti-CD28 antibodies was enough to induced the large inhibition of Kv1.3, when applied simultaneously.

Kv1.3 current regulation in p56lck tyrosine kinase deficient JCaM.1 cells

It has been reported that co-stimulation of CD3/CD28 induces more tyrosine phosphorylation and more cell proliferation than CD3 alone [2,16] and that Kv1.3 channel can be phosphorylated by p56lck kinase [17]. The inhibition of Kv1.3 current by Fas stimulation was not detected in p56lck-kinase deficient Jurkat cells, JCaM1 [12].

We examined effects of CD3/CD28 stimulation on Kv1.3 current in JCaM1. Whole-cell outward currents upon depolarization to +40 mV in JCaM.1 exhibited kinetics typical for Kv1.3 channels and were sensitivity to marigatoxin in the similar manner as in E6-1 (Fig. 3A). The Kv1.3 current density at the peak was 15.2 ± 2.7 pA/pF ($n = 6$) and tended to be but not significantly smaller than that in E6-1 ($p > 0.05$ vs. E6-1). Unexpectedly, sequential application of 10 μg/ml anti-CD3 antibody and 10 μg/ml anti-CD28 antibody decreased Kv1.3 current in JCaM1 in an additive fashion in the same manner as in E6-1. These results strongly suggest that p56lck kinase is not involved in the inhibition of Kv1.3 current by either CD3 or CD3/CD28 stimulation.

Kv1.3 current regulation by tyrosine phosphatase, CD45

We next examined effects of a phosphatase inhibitor, bpV(pic), on Kv1.3 current. Application of 3 μM bpV(pic) did not change Kv13. current activated at +40 mV for over 10 min (not shown in figure). The peak current density of Kv1.3 in E6-1 in the presence of 3 μM bpV(pic) was 22.1 ± 8.6 pA/pF ($n = 5$) and not significantly different from that in the absence (31.7 ± 11.5 pA/pF, $n = 9$, $p > 0.05$). The application of 10 μg/ml anti-CD3 antibody in the presence of 3 μM bpV(pic) inhibited Kv1.3 currents in a similar manner as in its absence. However, the addition of 10 μg/ml anti-CD28 antibody did not exhibit further Kv1.3 current inhibition in E6-1 (Fig. 4C). This may suggest that the additive inhibition by CD28 stimulation is mediated by activation of protein tyrosine phosphatases.

To elucidate further this possibility, effects of anti-CD3/CD28 antibodies on Kv1.3 were examined in J45.01, which is a mutant Jurkat cell line lacking CD45. The whole-cell outward currents elicited at +40 mV exhibited kinetics typical for Kv1.3 channels and sensitivity to margatoxin in the same manner as in E6-1. The peak current density was 23.7 ± 7.9 pA/pF ($n = 5$, $p > 0.05$ vs. E6-1 and JCaM1) (Fig. 4A). The CD3 stimulation in J45.01 decreased Kv1.3 current in the same manner as in E6-1. The additive inhibition of Kv1.3 current by anti-CD28 antibody was, however, not significant in J45.01 (Fig.4B and C). Moreover, additive inhibition of Kv1.3 current by anti-CD28 antibody in the presence of anti-CD3 antibody was not detected in the presence of 3 μM bpV(pic).

Discussion

It is clearly shown in the present study that CD3 stimulation alone reduced MgTx sensitive Kv1.3 current and that CD3/CD28 co-stimulation further reduced Kv1.3 current in Jurkat T cell. It has been well established that CD3 stimulation alone is not complete but that co-stimulation of both CD3 and CD28 is required for T lymphocyte fully activation [1,2]. In the long-term effects following T lymphocyte activation, the Kv1.3 channel expression

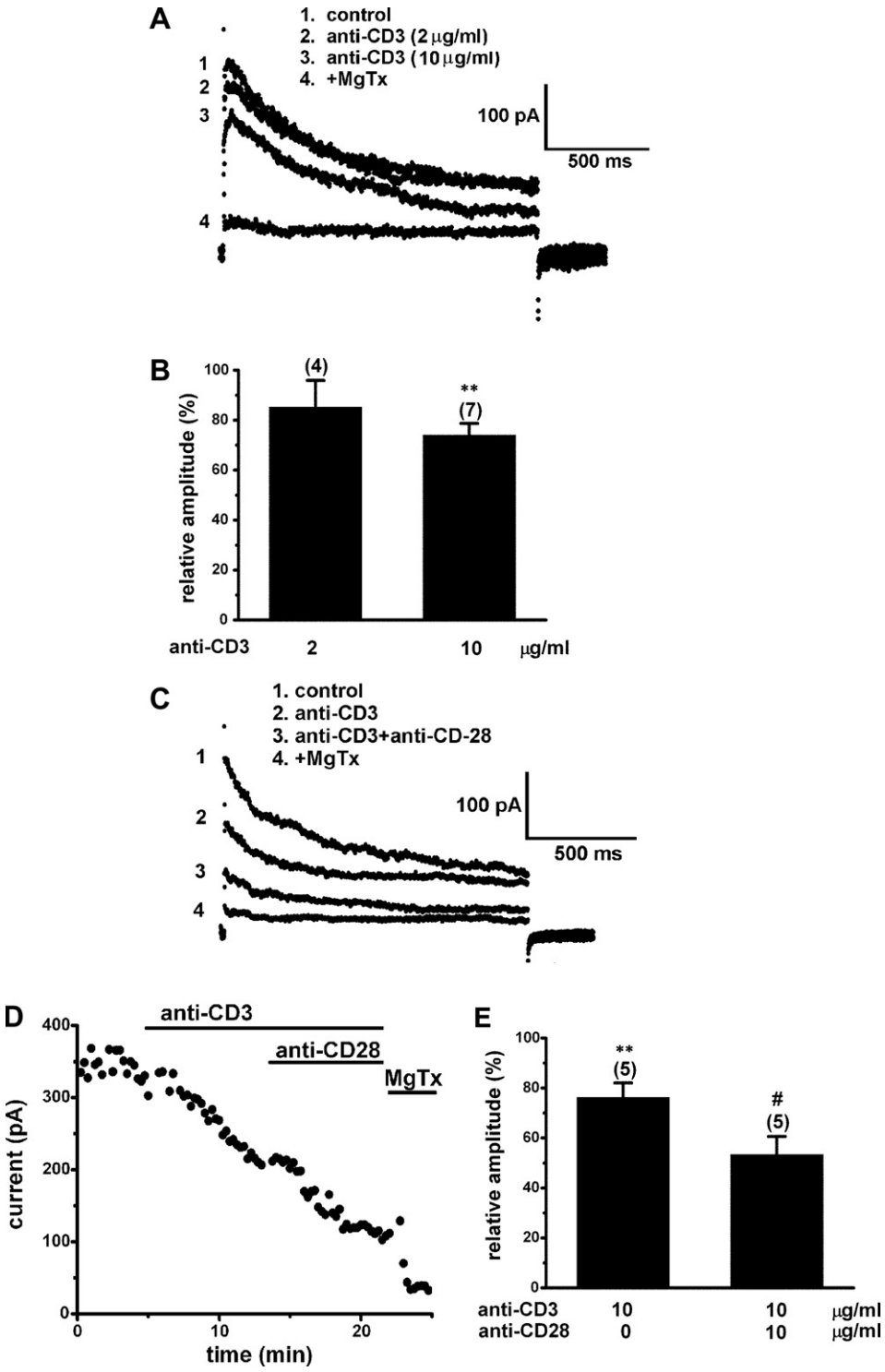

Fig. 1. Effects of anti-CD3 antibody or both anti-CD3 and anti-CD28 antibodies on Kv1.3 current in Jurkat cells. (A) Effects of anti-CD3 antibody on Kv1.3 current in Jurkat cells. Representative traces were obtained in Jurkat cells. Kv1.3 currents were elicited by the depolarization pulse of 1500-ms duration at 15-s intervals from the holding potential of −80 mV to +40 mV. The traces numbered from 1 to 4 in the figure were obtained under control conditions, in the presence of 2 or 10 μg/ml anti-CD3 antibody and after the application of 10 nM MgTx, respectively, as shown in the figure. (B) Summarized data about effects of anti-CD3 antibody on Kv1.3 current in Jurkat cells. The figure shows relative amplitude of peak Kv1.3 current at +40 mV in the presence of 2 and 10 μg/ml anti-CD3 antibody. Relative currents were expressed as percent of the margatoxin-sensitive current components versus that before antibody application. Data were shown as means ± SEM. The numbers in the parentheses above columns indicate numbers of cells examined. $^{**}p < 0.01$ vs. 100%. (C) Effects of 10 μg/ml anti-CD3 antibody and addition of 10 μg/ml anti-CD28 antibody on Kv1.3 currents elicited by depolarization to +40 mV in Jurkat cells. The traces numbered from 1 to 4 were obtained under conditions indicated correspondingly by numbers in the figure. (D) Time course of Kv1.3 current inhibition by anti-CD3 and CD28 antibodies and MgTx in Jurkat cells. The peak amplitude of outward currents at +40 mV was plotted against time. The treatments with 10 μg/ml anti-CD3 antibody, 10 μg/ml anti-CD28 antibody and 10 nM MgTx are shown by horizontal bars, respectively. (E) Summarized data about effects of 10 μg/ml anti-CD3 and addition of 10 μg/ml anti-CD28 antibodies on Kv1.3 current. $^{**}p < 0.01$ vs. 100%; $^{\#}p < 0.05$ vs. anti-CD3 antibody alone.

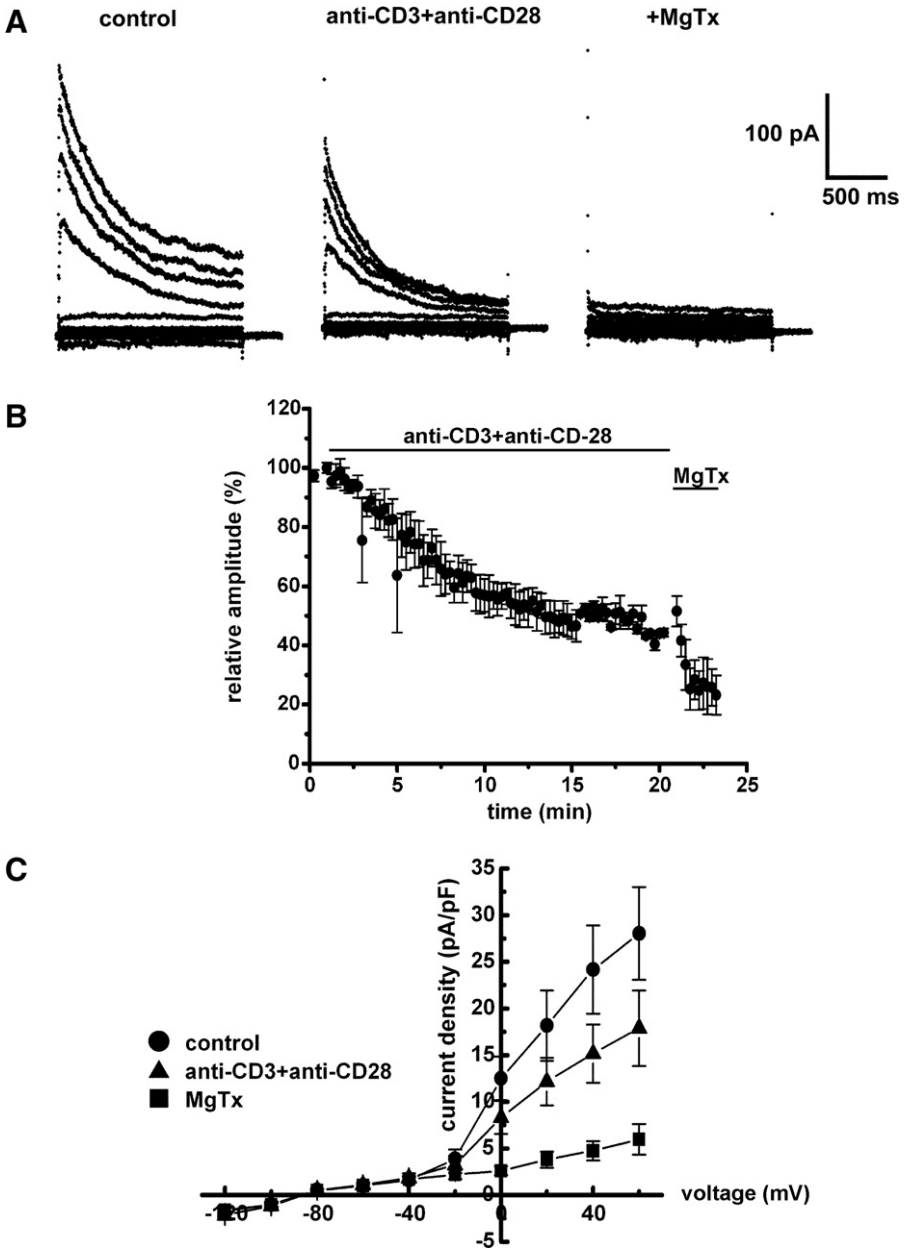

Fig. 2. Effects of simultaneous CD3/CD28 stimulation on Kv1.3 currents in Jurkat cells. (A) Representative traces of membrane currents were obtained by voltage-jump from −80 to test potentials in the range of −120 and +60 mV by 20 mV steps. (Left panel) Currents before application of antibodies. (Middle panel) Currents measured 20 min after simultaneous application of 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28 antibodies. (Right panel) Currents after application of 10 nM MgTx. (B) Time course of Kv1.3 current inhibition by simultaneous application of anti-CD3 and CD28 antibodies in Jurkat cells. Data were shown as means ± SEM (n = 5). Peak Kv1.3 current at +40 mV was expressed as percent of respective MgTx-sensitive current before application of antibodies at 0 min in each cell. (C) Current–voltage relationships of Kv1.3 currents between −120 and +60 mV in Jurkat cells. Peak outward currents under control conditions (●), 20 min after simultaneous application of anti-CD3 and anti-CD28 antibodies (▲) and after application of margatoxin (■) were normalized for cell capacitance, and were plotted against test potentials. n = 5.

is highly enhanced and this promotes Ca^{2+} entry and T cell proliferation [1,3]. In contrast, the inhibition of Kv1.3 current occurs as an acute response to CD3 stimulation and CD3/CD28 co-stimulation as shown in this study, and this may presumably prevent an excess Ca^{2+} entry from extracellular space through CRAC channels during a short time and protect cells from Ca^{2+} overload.

In the present study, the inhibitory effects of CD3 stimulation alone and CD3/CD28 co-stimulation on Kv1.3 current were still observed in p56lck-kinase deficient cells. These were rather unexpected results, since Kv1.3 channel inhibition and its phosphorylation by anti-Fas antibody and ceramide were abolished by the application of src kinase inhibitor herbimycin A or the deficiency of the p56lck tyrosine kinase [12,17]. The src kinase inhibitor PP2 abolished the hypoxia-induced inhibition of Kv1.3 channels in primary human T lymphocytes, and Kv1.3 channel sensitivity to hypoxia was lost in p56lck-deficient JCaM1 cells [18]. Moreover, CD28 stimulation regulates p56lck tyrosine kinase activity in T lymphocyte [16,19]. In contrast to these preceded findings, the present results suggest that p56lck is not or only partly involved in Kv1.3 channel inhibition by CD3 stimulation alone and CD3/CD28 co-stimulation.

The human homolog of *Drosophila* discs large (hDlg), which belongs to a family of MAGUKs recruited p56lck to the cytoplasmic domain of Kv1.3 channel in Jurkat T cells [20]. The hDlg is known as a widely expressed scaffold protein implicated in the organization of multi-protein complexes at cell adhesion site

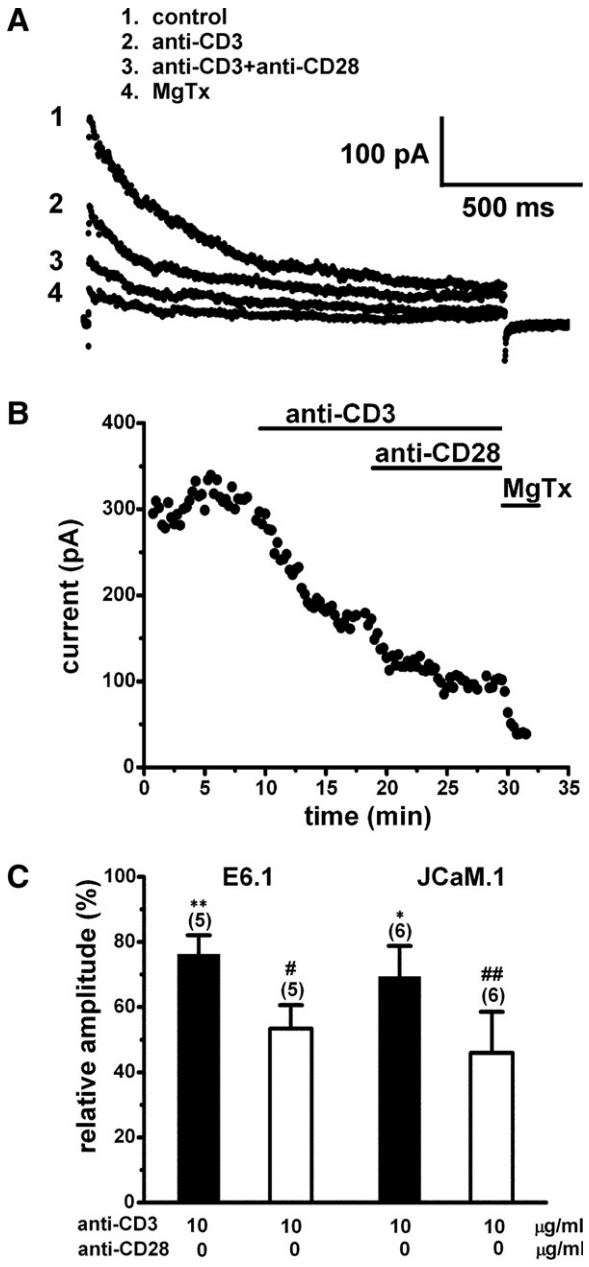

Fig. 3. Effects of anti-CD3 and anti-CD28 antibodies on Kv1.3 current in p56lck tyrosine kinase deficient Jurkat cells, JCaM.1. (A) Representative traces shown were obtained in JCaM.1 cells. Outward currents were elicited by depolarization from −80 to +40 mV under control conditions (1), in the presence of 10 μg/ml anti-CD3 antibody (2), in the presence of 10 μg/ml anti-CD3 and anti-CD28 antibodies (3) and after application of 10 nM MgTx (4), as shown correspondingly in the figure. (B) Time course of outward current inhibition by anti-CD3 and -CD28 antibodies and 10 nM MgTx in JCaM.1 cells. Peak outward currents at +40 mV were plotted against time. (C) Summarized data about effects of 10 μg anti-CD3 antibody and addition of 10 μM anti-CD28 antibody on relative peak amplitude of MgTx-sensitive currents at +40 mV in Jurkat E6.1 cells and JCaM.1 cells. The numbers in the parentheses above columns indicate numbers of cells examined. * and **: $p < 0.05$ and $p < 0.01$ vs. 100%, respectively. # and ##: $p < 0.05$ and $p < 0.01$ vs. anti-CD3 antibody alone in E6.1 cells and anti-CD3 antibody alone in JCaM.1, respectively.

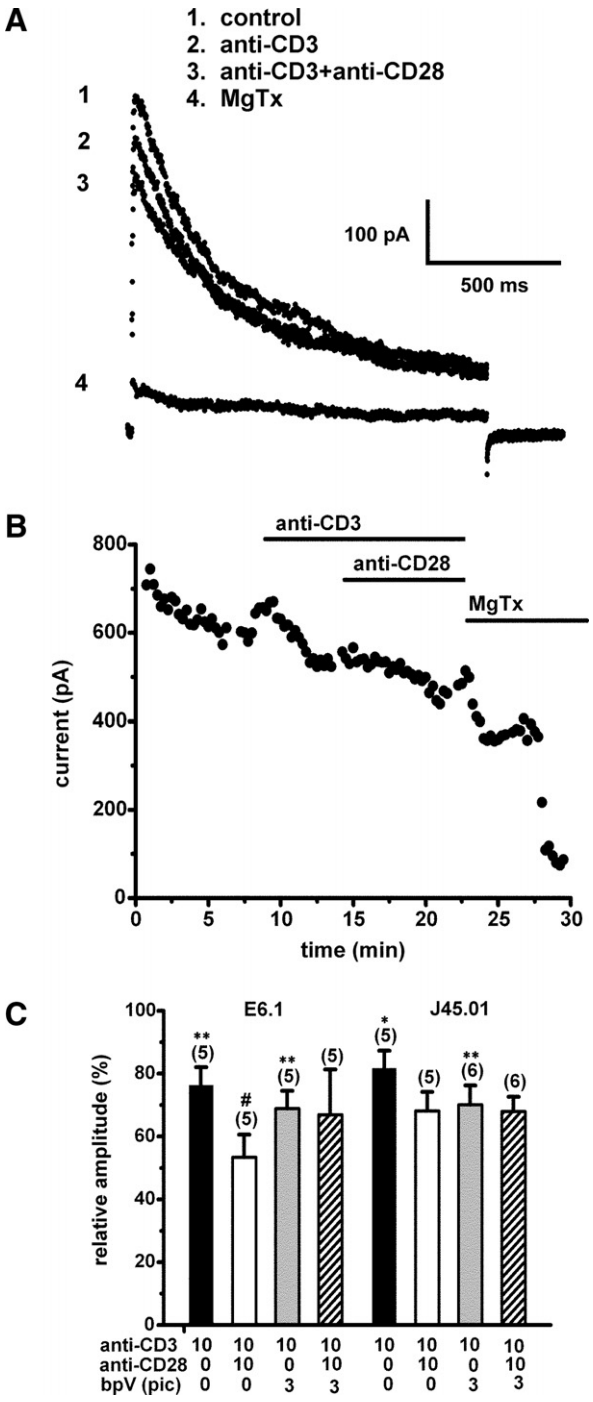

Fig. 4. Effects of anti-CD3 and anti-CD28 antibodies and bpV(pic) on Kv1.3 current in CD45 protein tyrosine phosphatase deficient Jurkat cells, J45.01 in comparison with control Jurlat T cells, E6.1. (A) Representative traces shown were obtained in J45.01 cells. Outward currents were elicited by depolarization from −80 to +40 mV under control conditions (1), in the presence of 10 μg/ml anti-CD3 antibody (2), in the presence of 10 μg/ml anti-CD3 and anti-CD28 antibodies (3) and after application of 10 nM MgTx (4), as shown correspondingly in the figure. (B) Time course of outward current inhibition by anti-CD3 and -CD28 antibodies and 10 nM MgTx in J45.01 cells. Peak outward currents at +40 mV were plotted against time. (C) Summarized data about effects of 10 μg anti-CD3 antibody, addition of 10 μM anti-CD28 antibody and bpV(pic) on relative peak amplitude of MgTx-sensitive currents at +40 mV in Jurkat E6.1 cells and J45.01 cells. The numbers in the parentheses above columns indicate numbers of cells examined. * and **: $p < 0.05$ and $p < 0.01$ vs. 100%, respectively. #$p < 0.05$ vs. anti-CD3 antibody alone in E6.1 cells.

[21]. Of interest is that the complete deficiency of p56lck in JCaM1 cells does not affect the interaction of hDlg with Kv1.3 channel [20]. These findings may support the possibility that p56lck does not contribute to Kv1.3 channel regulation by CD3 stimulation in the immunological synapse, in which Kv1.3 channels are clustered with hD1g [22].

A line of evidence in this study indicates that the additive reduction of Kv1.3 current by CD3/CD28 co-stimulation is mediated by CD45-phosphatase activity. The regulation of Kv1.3 chan-

nel function by protein tyrosine phosphatase (PTP) is first shown in this study. CD45 is essential for normal development of lymphocytes and their functional activity. In human and mice, the deficiency of CD45 exhibits severe immunodeficiency [23,24] and point mutations of CD45 have been associated with autoimmune and infectious diseases [25]. CD45 constitutively dephosphorylates the negative regulatory tyrosine of p56lck, Tyr^{505} and induces T cell activation. Interestingly, the reduction of Kv1.3 current by CD3 stimulation in this study was not significantly affected by the presence of bpV(pic), suggesting that tyrosine phosphorylation of Kv1.3 may not contribute significantly to its current reduction following CD3 stimulation. In J45.01, intracellular Ca^{2+} elevation is smaller than in Jurkat cells [26], suggesting that CD45 and other PTP positively regulate intracellular Ca^{2+}. Further study is required to elucidate the exact mechanisms underlying the contribution of CD45 to the additive reduction of Kv1.3 activity by CD3/CD28 co-stimulation.

The present study provides evidence indicating that CD3 stimulation and CD3/CD28 co-stimulation acutely down-regulate Kv1.3 channel activity in Jurkat cells. The involvement of p56lck in the down-regulation appeared to be limited, if any. The down-regulation of Kv1.3 activity by CD3/CD28 co-stimulation is mediated by the activation of CD45 protein tyrosine phosphatases.

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References

[1] R.E. Dolmetsch, K. Xu, R.S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, Nature 392 (1998) 933–936.
[2] A. Viola, S. Schroeder, Y. Sakakibara, A. Lanzavecchia, T lymphocyte costimulation mediated by reorganization of membrane microdomains, Science 283 (1999) 680–682.
[3] S. Feske, Calcium signalling in lymphocyte activation and disease, Nat. Rev. Immunol. 7 (2007) 690–702.
[4] H. Wulff, P.A. Calabresi, R. Allie, S. Yun, M. Pennington, C. Beeton, K.G. Chandy, The voltage-gated Kv1.3 $K^{(+)}$ channel in effector memory T cells as new target for MS, J. Clin. Invest. 111 (2003) 1703–1713.
[5] E.M. Gallo, K. Cante-Barrett, G.R. Crabtree, Lymphocyte calcium signaling from membrane to nucleus, Nat. Immunol. 7 (2006) 25–32.
[6] K.G. Chandy, H. Wulff, C. Beeton, M. Pennington, G.A. Gutman, M.D. Cahalan, K+ channels as targets for specific immunomodulation, Trends Pharmacol. Sci. 25 (2004) 280–289.
[7] T.E. DeCoursey, K.G. Chandy, S. Gupta, M.D. Cahalan, Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis?, Nature 307 (1984) 465–468
[8] L. Hu, M. Pennington, Q. Jiang, K.A. Whartenby, P.A. Calabresi, Characterization of the functional properties of the voltage-gated potassium channel Kv1.3 in human $CD4^+$ T lymphocytes, J. Immunol. 179 (2007) 4563–4570.
[9] C. Beeton, H. Wulff, N.E. Standifer, P. Azam, K.M. Mullen, M.W. Pennington, A. Kolski-Andreaco, E. Wei, A. Grino, D.R. Counts, P.H. Wang, C.J. LeeHealey, B.S. Andrew, A. Sankaranarayanan, D. Homerick, W.W. Roeck, J. Tehranzadeh, K.L. Stanhope, P. Zimin, P.J. Havel, S. Griffey, H.G. Knaus, G.T. Nepom, G.A. Gutman, P.A. Calabresi, K.G. Chandy, Kv1.3 channels are a therapeutic target for T cell-mediated autoimmune diseases, Proc. Natl. Acad. Sci. USA 103 (2006) 17414–17419.
[10] C. Beeton, H. Wulff, J. Barbaria, O. Clot-Faybesse, M. Pennington, D. Bernard, M.D. Cahalan, K.G. Chandy, E. Beraud, Selective blockade of T lymphocyte $K^{(+)}$ channels ameliorates experimental autoimmune encephalomyelitis a model for multiple sclerosis, Proc. Natl. Acad. Sci. USA 98 (2001) 13942–13947.
[11] C.M. Fanger, H. Rauer, A.L. Neben, M.J. Miller, H. Wulff, J.C. Rosa, C.R. Ganellin, K.G. Chandy, M.D. Cahalan, Calcium-activated potassium channels sustain calcium signaling in T lymphocytes. Selective blockers and manipulated channel expression levels, J. Biol. Chem. 276 (2001) 12249–12256.
[12] I. Szabo, E. Gulbins, H. Apfel, X. Zhang, P. Barth, A.E. Busch, K. Schlottmann, O. Pongs, F. Lang, Tyrosine phosphorylation-dependent suppression of a voltage-gated K^+ channel in T lymphocytes upon Fas stimulation, J. Biol. Chem. 271 (1996) 20465–20469.
[13] K.K. Cook, D.A. Fadool, Two adaptor proteins differentially modulate the phosphorylation and biophysics of Kv1.3 ion channel by SRC kinase, J. Biol. Chem. 277 (2002) 13268–13280.
[14] Y. Imaizumi, K. Muraki, M. Watanabe, Ionic currents in single smooth muscle cells from the ureter of the guinea-pig, J. Physiol. 411 (1989) 131–159.
[15] L.M. Helms, J.P. Felix, R.M. Bugianesi, M.L. Garcia, S. Stevens, R.J. Leonard, H.G. Knaus, R. Koch, S.G. Wanner, G.J. Kaczorowski, R.S. Slaughter, Margatoxin binds to a homomultimer of $K_{(V)}1.3$ channels in Jurkat cells. Comparison with $K_{(V)}1.3$ expressed in CHO cells, Biochemistry 36 (1997) 3737–3744.
[16] R. Tavano, G. Gri, B. Molon, B. Marinari, C.E. Rudd, L. Tuosto, A. Viola, CD28 and lipid rafts coordinate recruitment of Lck to the immunological synapse of human T lymphocytes, J. Immunol. 173 (2004) 5392–5397.
[17] E. Gulbins, I. Szabo, K. Baltzer, F. Lang, Ceramide-induced inhibition of T lymphocyte voltage-gated potassium channel is mediated by tyrosine kinases, Proc. Natl. Acad. Sci. USA 94 (1997) 7661–7666.
[18] P. Szigligeti, L. Neumeier, E. Duke, C. Chougnet, K. Takimoto, S.M. Lee, A.H. Filipovich, L. Conforti, Signalling during hypoxia in human T lymphocytes—critical role of the src protein tyrosine kinase p56Lck in the O_2 sensitivity of Kv1.3 channels, J. Physiol. 573 (2006) 357–370.
[19] A.D. Holdorf, K.H. Lee, W.R. Burack, P.M. Allen, A.S. Shaw, Regulation of Lck activity by CD4 and CD28 in the immunological synapse, Nat. Immunol. 3 (2002) 259–264.
[20] T. Hanada, L. Lin, K.G. Chandy, S.S. Oh, A.H. Chishti, Human homologue of the *Drosophila* discs large tumor suppressor binds to p56lck tyrosine kinase and Shaker type Kv1.3 potassium channel in T lymphocytes, J. Biol. Chem. 272 (1997) 26899–26904.
[21] A. Fujita, Y. Kurachi, SAP family proteins, Biochem. Biophys. Res. Commun. 269 (2000) 1–6.
[22] J. Matko, K^+ channels and T-cell synapses: the molecular background for efficient immunomodulation is shaping up, Trends Pharmacol. Sci. 24 (2003) 385–389.
[23] K.F. Byth, L.A. Conroy, S. Howlett, A.J. Smith, J. May, D.R. Alexander, N. Holmes, CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development in the selection of $CD4^+CD8^+$ thymocytes, and B cell maturation, J. Exp. Med. 183 (1996) 1707–1718.
[24] E.Z. Tchilian, D.L. Wallace, R.S. Wells, D.R. Flower, G. Morgan, P.C. Beverley, A deletion in the gene encoding the CD45 antigen in a patient with SCID, J. Immunol. 166 (2001) 1308–1313.
[25] R. Dawes, S. Petrova, Z. Liu, D. Wraith, P.C. Beverley, E.Z. Tchilian, Combinations of CD45 isoforms are crucial for immune function and disease, J. Immunol. 176 (2006) 3417–3425.
[26] V. Imbert, J.F. Peyron, D. Farahi Far, B. Mari, P. Auberger, B. Rossi, Induction of tyrosine phosphorylation and T-cell activation by vanadate peroxide an inhibitor of protein tyrosine phosphatases, Biochem. J. 297 (1994) 163–173.