Modulation of Endoplasmic Reticulum Calcium Pump Expression during T Lymphocyte Activation*

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Calcium mobilization from intracellular storage organelles is a key component of the second messenger system inducing cell activation. Calcium transport ATPases associated with intracellular calcium storage organelles play a major role in controlling this process by accumulating calcium from the cytosol into intracellular calcium pools. In this study the modulation of the expression of the sarco-endoplasmic reticulum calcium transport ATPase (SERCA) isoenzymes has been studied in lymphocytes undergoing phorbol myristate acetate and ionomycin-induced activation. In several T lymphocyte cell lines a combined treatment by the two drugs resulted in an approximately 90% decrease of the expression of the calcium pump isofrom recognized by the PLIM430 isofrom-specific antibody, whereas the expression of the SERCA 2b isofrom was increased approximately 2-fold. Phorbol ester or ionomycin applied separately was ineffective. In Jurkat T cells the down-modulation of expression of the SERCA isoform recognized by the PLIM430 antibody appeared concomitantly with the induction of interleukin-2 expression and could be inhibited by the immunosuppressant drug cyclosporine-A. These data indicate that T cell activation induces a selective and cyclosporine-A-sensitive modulation of the expression of the SERCA calcium pump isoforms. This reflects a profound reorganization of the calcium homeostasis of differentiating cells and in the pharmacological modulation of lymphocyte function.

Calcium as a second messenger is a key component of the cellular signaling network controlling lymphocyte function (Refs. 1–3 and references therein). Activation of the T cell receptor complex and associated coreceptors by antigen presentation leads to the formation of two second messengers, diacylglycerol and inositol 1,4,5-trisphosphate (IP3). The for-
was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

The Jurkat-derived JurE6–1 clone (22), as well as the Molt-4 and the CCRF-CEM cell lines were obtained from ATCC (Rockville, MD). Cells were grown in RPMI 1640 medium with Glutamax-I supplemented with 10% heat-inactivated fetal calf serum and 2 mM glutamine (Life Technologies, Inc.) in a humidified cell culture incubator at 37 °C in an atmosphere of 95% air and 5% CO2. Glutamax-I and glutamine were used in combination to compensate for the increased metabolic requirements of the cells due to the experimentally induced increase of calcium permeability.

Cell Treatments—Exponentially growing cells were harvested by centrifugation, resuspended in fresh complete medium at a density of 2 x 10^5 cells/ml and placed in 9-cm-diameter Petri dishes. Cells were then placed for 1 h in the cell culture incubator before stimulation. Drugs were then added from concentrated stock solutions in Me2So. The concentration of Me2So did not exceed 0.1%, was included in control experiments, and did not interfere with the assays. Cyclosporine-A was added 1 h prior to PMA or ionomycin treatment.

Following the treatments for the time periods indicated on the figures, cell counts and viabilities were determined by the trypan blue exclusion method, and the cells were harvested by centrifugation. The supernatant was saved for IL-2 determination, and the cells were resuspended in ice-cold phosphate-buffered saline, spun down in microcentrifuge tubes, and immediately frozen as a pellet on dry ice. The cell pellets were thawed at a density of 10^7 cells/ml in a lysis buffer containing 62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 5 mM EDTA, 100 mM dithiothreitol, 2 mM urea, and 0.02% bromphenol blue (23). Cells were homogenized by aspiration (15–20 strokes) using a 2-ml Hamilton syringe.

Samples containing the lysates of 2 x 10^5 cells (20 μl) were run on 7.5% alkaline Laemmli-type polyacrylamide gels and transferred onto nitrocellulose membranes. Saturation of nitrocellulose and immunostaining was performed in a buffer containing 10 mM Tris, pH 7.4, 150 mM NaCl, 5% dry milk, and 0.1% Tween 20 as described previously (24). Luminoseent signal was generated and detected using the Enhanced Chemiluminescence system (ECL) of Amersham. Luminograms were scanned and quantitated using an LKB Ultrascan XL Laser Densitometer. In control experiments we determined that the conditions used for immunostaining and detection gave signals proportional to the amount of SERCA protein loaded on the gels, and thus these conditions were suitable for the quantitative detection of SERCA protein in whole cell lysates. Immunofluorescent staining was performed on acetone-fixed cells as described previously (25).

SERCA_PLM430 and SERCA 2b mRNA levels were estimated using a semiquantitative reverse transcriptase-PCR method described previously (48). Briefly, total RNA from cells treated for 4 days with PMA plus ionomycin or vehicle control was reverse transcribed and amplified using the Perkin-Elmer GeneAmp RNA PCR kit and Taq DNA polymerase according to the manufacturers instructions by 30 cycles (each cycle consisting of 30 s at 94 °C, 2 min at 55 °C, and 2 min at 72 °C). The primers used to amplify SERCA 2b were TCATCTTCCAGATCCACCGCT and GTCAAGACCAGAACATATC, which cover the region from bases 3000 to 3074 (17). Data presented in this paper represent the results of at least four independent experiments.

RESULTS

Down-regulation of SERCA_PLM430 Expression by PMA Plus Ionomycin—JurE6–1 cells were treated for 4 days with 0.5 μM ionomycin, 10 nM PMA, or a combination of the two drugs. Whole cell lysates were electrophoresed and immunoblotted using the SERCA isoform-specific discriminating antibodies IID8 and PLIM430. IL-2 secretion by the cells was quantitated using anti-SERCA antibodies.

As shown on Fig. 1, untreated cells expressed similar amounts of the two pump isoforms (Fig. 1A, lanes 1 and 5), whereas a combined treatment with the two drugs resulted in an approximately 90% decrease of the expression of SERCA_PLM430 (Fig. 1, A, lane 2, and B) and an approximately 2-fold increase of the expression of SERCA 2b (Fig. 1, A, lane 6, and lane 8).

FIG. 1. Selective modulation of SERCA_PLM430 and SERCA 2b expression by PMA plus ionomycin. JurE6–1 cells were treated for 4 days with Me2So vehicle, 10 nM PMA plus 0.5 μM ionomycin in combination, 10 nM PMA or 0.5 μM ionomycin. Whole cell lysates were electrophoresed and immunoblotted using isoform-specific discriminating anti-SERCA antibodies. A, immunoblots of cells treated with Me2So vehicle (lanes 1 and 5), PMA plus ionomycin (lanes 2 and 6), PMA (lanes 3 and 7), and ionomycin (lanes 4 and 8) immunostained with the SERCA_PLM430 and SERCA 2b, respectively. B, densitometric analysis of luminograms shown in A. Filled bars, SERCA_PLM430; empty bars, SERCA 2b. In cells treated with PMA together with ionomycin, the expression of the SERCA_PLM430 isoenzyme is decreased by 85%, and the expression of the SERCA 2b isoform is increased by a factor of 2.3 when compared with the untreated control. C, semiquantitative reverse transcription-PCR amplification of SERCA 2b (lanes 1 and 2) and of SERCA_PLM430 mRNA (lanes 3 and 4) in JurE6–1 cells treated with vehicle (lanes 1 and 3) or with PMA plus ionomycin (lanes 2 and 4) for 4 days. bp, base pairs.

B). Ionomycin or PMA, when applied alone, did not modify significantly the expression of either calcium pump isoform in this system (Fig. 1, A, lanes 3, 4, 7, and 8, and B). The differ-
ential modulation of the expression of the two pump species was also manifest on the mRNA level. As shown on Fig. 1C, treatment of JurE6–1 cells by PMA plus ionomycin resulted in an approximately 2-fold decrease of SERCA PLIM430 mRNA (lane 3, untreated; lane 4, treated), whereas in the same cells SERCA 2b mRNA was increased approximately 2-fold (lanes 1 and 2).

Induction of Interleukin-2 Synthesis—Supernatants of JurE6–1 cells treated as above were collected, and their IL-2 content was quantitated by ELISA. In accordance with previous data (22, 26–30), a combined treatment by PMA together with ionomycin resulted in a marked induction of IL-2 synthesis by the cells (1.2 ng/10⁵ cells at day 4). In accordance with data in the literature (22, 26, 29, 30), PMA or ionomycin, when applied alone, did not induce detectable IL-2 synthesis (i.e., less than 3 pg/10⁵ cells).

Time Course of SERCAPLIM430 Down-modulation—JurE6–1 cells were treated for various time periods with PMA together with ionomycin, and SERCA expression was determined by immunoblotting. As shown on Fig. 2A and B, SERCA PLIM430 expression started to decline at hour 9 after treatment. The induction of SERCA 2b expression followed a somewhat slower time course, being manifest starting at day 2 (Fig. 2A and C).

The state of activation of the cells was monitored in parallel by immunostaining for the α chain of the IL-2 receptor and by measuring IL-2 secretion, two established markers of T lymphocyte activation. SERCA PLIM430 down-regulation and the induction of IL-2 synthesis (Fig. 2E) followed a similar time course, whereas the expression of the α chain of the IL-2 receptor expression appeared somewhat delayed (Fig. 2, A and D). In accordance with previous data (31), PMA plus ionomycin treatment resulted in growth arrest of Jurkat cells with maintained viability.

Immunofluorescent Staining—SERCA PLIM430 down-regulation by PMA together with ionomycin was also manifested at the single cell level. In untreated cells a granular staining was seen for both SERCA isoforms in the cytoplasmic space, corresponding to the endoplasmic reticulum (Fig. 3, A and C). Although the fluorescence signal for SERCA 2b was not detectably modified by a treatment with PMA plus ionomycin (Fig. 3B), a marked decrease of staining for SERCA PLIM430 could be seen in PMA plus ionomycin-treated cells (Fig. 3D).

Effect of the Immunosuppressant Cyclosporine-A—JurE6–1 cells were preincubated with various concentrations of cyclosporine-A for 1 h and then treated for 4 days with PMA and ionomycin. As shown on Fig. 4, cyclosporine-A in the submicromolar range abolished in a concentration-dependent manner the modulation of the expression of SERCA PLIM430 (Fig. 4A) and of SERCA 2b (Fig. 4B) as well as IL-2 synthesis (Fig. 4C) induced by PMA plus ionomycin. Cyclosporine-A applied alone had no effect on SERCA expression (not shown).

DISCUSSION

Second messenger mediated calcium mobilization and protein kinase C activation are key events in lymphocyte signaling. In this work these signals were induced simultaneously by
the combined treatment of cells by ionomycin and PMA. This technique is widely employed in the literature to obtain T cell activation and induces IL-2 synthesis and the expression of the \( \alpha \) chain of the IL-2 receptor (22, 26–30, 32, 33).

As shown in the present work, such a treatment results in the isoform-specific modulation of the expression of the SERCA enzymes expressed in Jurkat T lymphoblastoid cells. The SERCA
\textsuperscript{PLIM430} calcium pump isoform is almost completely down-modulated, whereas the expression of SERCA 2b is increased 2-fold in the same cells. This results in a more than 15-fold overall increase in the relative ratio of the expression of SERCA 2b versus SERCA
\textsuperscript{PLIM430}. Similar results were obtained with Molt-4 and CCRF-CEM T lymphocytes as well (not shown).

The modulation of SERCA
\textsuperscript{PLIM430} expression in Jurkat cells occurred in parallel with the induction of IL-2 secretion and preceded the induction of the expression of the \( \alpha \) chain of the IL-2 receptor. Similarly to the induction of IL-2 expression (22, 26), the effect on SERCA expression was strictly dependent on the simultaneous presence of both drugs, because PMA or ionomycin, when applied alone, was without effect either on SERCA expression or on IL-2 expression.

Cyclosporine-A is a major, clinically used immunosuppressant that forms trimeric complexes with cyclophyllins and the calcium-calmodulin-dependent serin-threonin phosphatase, calcineurin. The formation of such a ternary complex leads to the inhibition of calcineurin enzyme activity and impaired signal transduction to the nucleus by NFAT (39–37). In this work the PMA plus ionomycin-induced IL-2 secretion and the modulation of SERCA 2b and SERCA
\textsuperscript{PLIM430} expression were abolished by cyclosporine-A in the submicromolar range. This suggests that calcineurin-dependent signaling, a key component in T cell activation, is involved in the control of the expression of SERCA enzymes in lymphocytes.

It has been shown earlier that lymphocyte activation produces modifications of the calcium storage and release characteristics of the cell (38) and that SERCA enzyme activity is involved in the control of cell proliferation and of lymphocyte activation (39–42). However, the role and the modulation of the expression of the various SERCA isoforms, coexpressed in the cell, have not been previously investigated. The data presented in this work indicate that striking differences exist in the regulation of expression of the SERCA isoforms during lymphocyte activation. This phenomenon probably reflects that various SERCA isoforms play functionally distinct roles and are associated with functionally distinct subcompartments of the endoplasmic reticulum.

SERCA
\textsuperscript{PLIM430} is believed to be a variant of SERCA 3, the expression of which is restricted to cells of hemopoietic origin (15–18). This enzyme, at least in platelets, is specifically associated with the IP\(_{3}\)–mobilizable calcium pool (20, 21, 43). Lymphocyte activation as well as apoptosis are strictly dependent on the mobilization of the IP\(_{3}\)–sensitive calcium pool (1, 8, 44–46). The down-modulation of SERCA
\textsuperscript{PLIM430} during activation may lead to decreased filling of this pool. Because the depletion of the IP\(_{3}\)–sensitive calcium pool via IP\(_{3}\)–induced calcium release (2, 6, 7) or by direct inhibition of SERCA activity by drugs such as thapsigargin (39, 40) is known to result in the generation of activatory signals, SERCA
\textsuperscript{PLIM430} down-modulation may contribute to the maintenance of the activated state or may alter the apoptotic potential of the cell.

The observation, shown in this paper, that SERCA
\textsuperscript{PLIM430} and SERCA 2b mRNA levels are differentially modulated by PMA plus ionomycin suggests that transcriptional regulation may be involved in the control of SERCA expression during lymphocyte activation. Indeed, simultaneous calcium mobilization and protein kinase C activation sets in motion a complex intracellular signaling network including calmodulin-dependent protein kinases and calcineurin (49), as well as the Jun-N-terminal kinase pathway (47). This leads to the modulation of the activity of several transcription factors such as NF\(\kappa\)B, NFAT or AP-1 (9), resulting in a complex set of modifications of gene expression, including, as shown in this work, intracellular calcium pump isoenzymes. The use of specific inhibitors of the different key factors of this complex signaling system and experiments addressing SERCA gene transcription and mRNA stability will permit a more detailed analysis of the mechanisms involved in the regulation of SERCA expression.

The plasticity of expression of the various SERCA isoforms upon cell activation represents a previously unrecognized level
of complexity of lymphocyte calcium homeostasis and shows that different SERCA isofoms and therefore presumably different calcium pools may play distinct roles in cell activation. This finding points at the dynamic nature of the structure and function of calcium homeostatic systems of differentiating cells and may open new perspectives in the understanding of intracellular calcium homeostasis and in the pharmacological control of lymphocyte function.

Acknowledgments—We are grateful to Dr. Agnes Enyedi, Dr. BalaS Sarkadi, Dr. Anna Berardi, and Dr. Ali Sab for helpful discussions. The help of Dr. Jacques Maclouf with the IL-2 ELISA experiments is acknowledged. We express special thanks to Prof. Neville Crawford for giving us the PL/IM430 hybridoma.

REFERENCES

1. Weiss, A., and Imboden, J. B. (1987) Advances Immunol. 41, 1–38
2. Premack, B. A., and Gardner, P. (1992) Am. J. Physiol. 263, C1119–C1140
3. Crabtree, G. R., and Clipstone, N. A. (1994) Annu. Rev. Biochem. 63, 1045–1083
4. Tsien, R. Y., Pozzan, T., and Rink, T. J. (1989) Nature 335, 68–71
5. Berridge, M. J. (1995) Nature 376, 315–325
6. Berridge, M. J. (1995) Biochem. J. 312, 1–11
7. Putney, J. W. (1996) Cell Calcium 11, 611–624
8. Pozzan, T., Rizzuto, R., Volpe, P., and Meldolesi, J. (1994) Physiol. Rev. 74, 595–636
9. Rothenberg, E., and Ward, S. B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9358–9365
10. Chen, D., and Rothenberg, E. V. (1993) J. Biol. Chem. 268, 149, 10750–10755
11. Leonard, W. J., Kroenke, M., Peffer, N. J., Depper, J. M., and Greene, W. C. (1991) J. Exp. Med. 174, 15024–15031
12. Korczak, B., Zarain-Herzberg, A., Brandl, C. J., Ingles, J., Green, N. M., and MacLennan, D. H. (1988) J. Biol. Chem. 263, 4813–4819
13. Lytton, J., and MacLennan, D. H. (1988) J. Biol. Chem. 263, 15024–15031
14. Wu, K. D., Lee, W. L., Wey, J., Bungard, D., and Lytton, J. (1995) Am. J. Physiol. 260, 775–784
15. Dode, L., Wuytack, F., Kools, P. F., Baba-Aissa, F., Raeymaekers, L., Brik, F., Van de Ven, W. J. M., and Casteels, R. (1995) Biochem. J. 318, 689–699
16. Lacabaratz, C., Corvazier, E., Kovacs, T., Bohe, R., Wuytack, F., Papp, B., and Enouf, J. (1996) Biochem. J. 318, 359–360
17. Papp, B., Enyedi, A., Kovacs, T., Sarkadi, B., Wuytack, F., Thastrup, O., Gardos, G., Bredoux, R., Levy-Toledano, S., and Enouf, J. (1991) J. Biol. Chem. 266, 14593–14596
18. Papp, B., Enyedi, A., Pasisz, K., Kovacs, T., Sarkadi, B., Gardos, G., Magzner, C., Wuytack, F., and Enouf, J. (1992) Biochem. J. 288, 297–302
19. Hack, N., Wilkinson, J. M., and Crawford, N. (1988) Biochem. J. 250, 355–361
20. Papp, B., Pasisz, K., Kovacs, T., Sarkadi, B., Gardos, G., Enouf, J., and Enyedi, A. (1993) Cell Calcium 14, 531–538
21. Engelder, S., Wolosker, H., and de Meis, L. (1995) J. Biol. Chem. 270, 21050–21055
22. Weiss, A., Wiscoeil, R., and Stobo, J. D. (1984) J. Immunol. 133, 123–128
23. Haim, R., Iwata, T., Zvaritch, E., Adano, H. P., Ratihauer, S., Schleger, E. E., Guerini, D., and Carafoi, E. (1992) J. Biol. Chem. 267, 24476–24484
24. Kovacs, T., Corvazier, E., Papp, B., Magner, C., Bredoux, R., Enyedi, A., Sarkadi, B., and Enouf, J. (1994) J. Biol. Chem. 269, 6177–6184
25. Harlow, E., and Lane, D. (1988) in Antibodies: A Laboratory Manual, pp. 359–420, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Truene, A., Albert, F., Gelstein, P., and Schmitt-Verhulst, A.-M. (1985) Nature 313, 318–320
27. Altman, M. M., Mally, I. M., and Isakov, N. (1992) ImmunoJ. Biol. Chem. 67, 645–471
28. Mattila, P. S., Ullman, K. S., Fiering, S., Emmel, E. A., McCutcheon, M., Crabtree, G. R., and Herzenberg, L. A. (1990) EMBO J. 9, 4425–4433
29. Lugo, C., Burgeon, E., Carew, J. A., McCaffrey, P. G., Badalian, T. M., Lane, W. S., Hagan, P. G., and Ras, A. (1996) Mol. Cell. Biol. 16, 3955–3966
30. Tsutsumi, A., Kubo, M., Fujii, H., Preire-Moor, J., Turck, C. W., and Ransome, J. T. (1993) J. Biol. Chem. 150, 1746–1754
31. Venkataraman, L., Burakoff, S. J., and Sen, R. (1995) J. Exp. Med. 181, 1091–1099
32. Wesselborg, S., Fruman D. A., Sogoo, K. J., Bierer, B. E., and Burakoff, S. J. (1996) J. Biol. Chem. 271, 1274–1277
33. Timmerman, L., Clipstone, N. A., Ho, S. N., Northrop, J. P., and Crabtree, G. R. (1996) Nature 383, 837–840
34. Shiiba, F., Price, E. R., Milan, D., and McKeon, F. (1996) Nature 382, 370–373
35. Lob, C., Shaw, K. T. Y., Carew, J., Viola, J. P. B., Luo, C., Perrino, B. A., and Rao, A. (1996) J. Biol. Chem. 271, 10884–10891
36. Holloway, M. P., and Bram, R. J. (1996) J. Biol. Chem. 271, 8549–8552
37. Clementi, E., Martin, G., Grimaldi, L., M., Brambilla, E., and Meldolesi, J. (1994) Eur. J. Immunol. 24, 1365–1371
38. Breittmayer, J.-P., Ticchioni, M., Ferrua, B., Bernard, A., and Asserl, C. (1993) Cell. Immunol. 149, 248–257
39. Papp, B., and Byrn, R. A. (1995) J. Biol. Chem. 270, 10278–10283
40. Cheng, G., Liu, B. P., Yu, Y., Dgiio, C., and Kuo, T. H. (1996) J. Biol. Chem. 329, 65–72
41. Waldron, R. T., Short, A. D., and Gill, D. L. (1995) J. Biol. Chem. 270, 11955–11961
42. Cavaillie, L., Coassin, M., and Alexandre, A. (1995) Biochem. J. 310, 449–452
43. Jayaraman, T., Ondriasova, E., Ondrias, K., Harnick, D. J., and Marks, A. R. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 6007–6011
44. Khan, A. A., Soloski, M. J., Sharp, A. H., Schilling, G., Sabatini, D. M., Li, S.-H., Ross, C. A., and Snyder, S. H. (1996) Science 273, 503–507
45. Jiang, S., Chow, S. C., Nicotera, P., and Orrenius, S. (1994) Exp. Cell Res. 212, 84–92
46. Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M., and Ben-Neirah, Y. (1994) Cell 77, 727–736
47. Boc, R., Bredoux, R., Wuytack, F., Quarrick, R., Kovacs, T., Papp, B., Corvazier, E., Magner, C., and Enouf, J. (1994) J. Biol. Chem. 269, 1417–1424
48. Enslen, H., and Soderling, T. R. (1994) J. Biol. Chem. 269, 20872–20877
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J. Biol. Chem. 1997, 272:10746-10750.
doi: 10.1074/jbc.272.16.10746

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