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STEAROYL-CoA DESATURASE GENE EXPRESSION IN LYMPHOCYTES

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SUMMARY: B lymphocytes from the spleens of normal (BALB/c) and autoimmune (MRL/*lpr*) strains of mice express the SCD-2 form of stearoyl-CoA desaturase as opposed to the SCD-1 form of the gene which is expressed in liver. However, whereas BALB/c T cells did not express SCD-1 or SCD-2, both BALB/c thymocytes and MRL/*lpr* T cells expressed SCD-2, suggesting a developmental down-regulation of SCD-2 within the T cell lineage. Northern analyses also revealed the expression of SCD-2 in the T cell lines BW5147, CTLL-2 and HT-2 and in BCL_1, a B cell line. SCD-1 expression was not detected in any of the lymphoid cells tested. Finally, we show that SCD-2 gene expression is inhibited by arachidonic acid (20:4). These results demonstrate the complexity of SCD-2 regulation in lymphoid cells.

Unsaturated fatty acids (UFAs) are either synthesized endogenously by the actions of stearoyl-CoA desaturase or may be derived from exogenous sources (1,2). Stearoyl-CoA desaturase catalyzes the insertion of a double bond between the C-9 and C-10 atoms of either palmitic (16:0) or stearic (18:0) acids (2,3). Recently, at least two genes encoding stearoyl-CoA desaturase have been identified (4,5). Whereas liver expressed SCD-1 exclusively, spleen, heart and brain expressed only SCD-2 (5). By contrast, both SCD-1 and SCD-2 were expressed in adipose tissue, lung and kidney to varying extents (5).

Previous studies have shown stearic acid (18:0) to be a potent inhibitor of T lymphocyte proliferation and function (6,7). The susceptibility of T lymphocytes to 18:0 was determined to be due to their unique deficiency in stearoyl-CoA desaturase gene expression (8,9). By contrast, B cells express stearoyl-CoA desaturase and are unaffected by exogenous 18:0. Such a fundamental difference between B and T lymphocytes indicates cell-type specific gene expression with regard to stearoyl-CoA desaturase.

In this report we provide additional information pertaining to the expression of stearoyl-CoA desaturase in B and T lymphocytes. First we show that lymphoid cells express the SCD-2 form of the gene as opposed to SCD-1. Second, based on studies with BALB/c thymocytes and MRL/*lpr* T cells, we define a developmental down-regulation of SCD-2 in T cells. Finally, in contrast to normal T lymphocytes, several T cell lines are shown to express SCD-2.

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MATERIALS AND METHODS

Isolation of Murine Lymphocytes and Thymocytes. Male and female BALB/c mice, 4-6 weeks of age, were obtained from an inbred colony maintained at East Carolina University School of Medicine. MRL/*lpr* mice (4-6 weeks) were obtained from Charles River Breeding Laboratories (Raleigh, NC). B and T lymphocytes were isolated as previously described (10). Thymus cell suspensions were obtained by gently pressing minced tissue through a stainless steel mesh. Erythrocytes and macrophages were depleted by NH_4Cl lysis and passage of cells over glass wool columns, respectively (10). The heterogeneous thymus population was then incubated in suspension with supernatants containing anti-CD4 (anti-L3T4) and anti-CD8 (anti-Lyt2.2) monoclonal antibodies produced by ATCC cell lines TIB 207 and TIB 210, respectively. Subsequent selection on rabbit anti-rat Ig-coated petri plates yielded an adherent thymic cell population consisting of $CD4^+8^-$, $CD4^-8^+$ and $CD4^+8^+$ cells (11).

Cell Culture. The T cell lines BW5147 (ATCC TIB 48), HT-2 (ATCC CRL 1841), CTLL-2 (ATCC TIB 214), and the B cell line, BCL_1 (ATCC TIB 197) were obtained from American Type Culture Collection. Unless otherwise stated, all cell lines were cultured in RPMI containing 10% FBS.

Flow Cytometry. Flow cytometric analyses were performed on a Becton-Dickinson FACS 440 flow cytometer with the laser tuned to an excitation wavelength of 488 nm at an output of 400 mW. Emission of fluorescence was assayed at 530 nm (fluorescein) and at 575 nm (phycoerythrin). Fluorescein isothiocyanate conjugated (FITC) goat anti-mouse polyvalent immunoglobulins (Sigma, St. Louis, MO), FITC-conjugated monoclonal anti-mouse Thy-1.2 (ICN, Costa Mesa, CA), FITC-anti-Lyt-2 (anti-CD8) and phycoerythrin (PE) conjugated anti-L3T4 (anti-CD4) (Becton Dickinson, San Jose, CA) were all used at a dilution of 1:100. Cell populations were stained and analyzed by flow cytometry as previously described (10).

RNase Protection Assays. Riboprobes specific for SCD-1 and SCD-2 were generated from cDNA constructs obtained from M.D. Lane (4,5). Riboprobes specific for mouse ß-actin were generated from DNA contained within an Ambion *in vitro* transcription kit. Radiolabeled ([α-^{32}P]UTP) antisense RNA probes were synthesized using T7 or SP6 RNA polymerases from linearized templates. Hybridizations were carried out using 8-10 x 10^4 cpm of labeled probes and 5 μg total cellular RNA in a solution containing 80% formamide at 42°C for 12 to 16 h (12). Following digestion with RNases A and T_1, protected RNA fragments were isolated by ethanol precipitation and separated by SDS-polyacrylamide gel electrophoresis in 5% acrylamide, 8M urea gels. Gel regions corresponding to the labeled RNA fragments were visualized by autoradiography.

Northern Blot Analyses. Expression of stearoyl-CoA desaturase mRNA was analyzed using a cDNA probe for rat liver stearoyl-CoA desaturase obtained from P. Strittmatter (13). Cell type specific expression of SCD-1 versus SCD-2 was assayed using probes provided by M.D. Lane (4,5); ß-actin was measured using a probe for chicken ß-actin purchased from Oncor (Gaithersburg, MD). Total cellular RNA was isolated as described (8). To induce liver stearoyl-CoA desaturase gene expression, BALB/c mice were fed a fat-free diet for a period of eight weeks (14). Liver RNA was isolated as above except that tissue was homogenized using a polytron. RNA from fully differentiated 3T3-L1 adipocytes was kindly provided by Drs. Pekala and Stephens (15). Northern analyses were performed as described previously (8); washed filters were subjected to autoradiography using Kodak XAR5 film in cassettes containing DuPont lightening plus intensifying screens for 1-3 days at -80°C.

RESULTS AND DISCUSSION

Previously, we have shown that BALB/c splenic B cells but not T cells express stearoyl-CoA desaturase (8). Since M.D. Lane and co-workers have recently shown that spleen cells express SCD-2 and not SCD-1 (5), it seemed likely that BALB/c B cells would also express SCD-2.

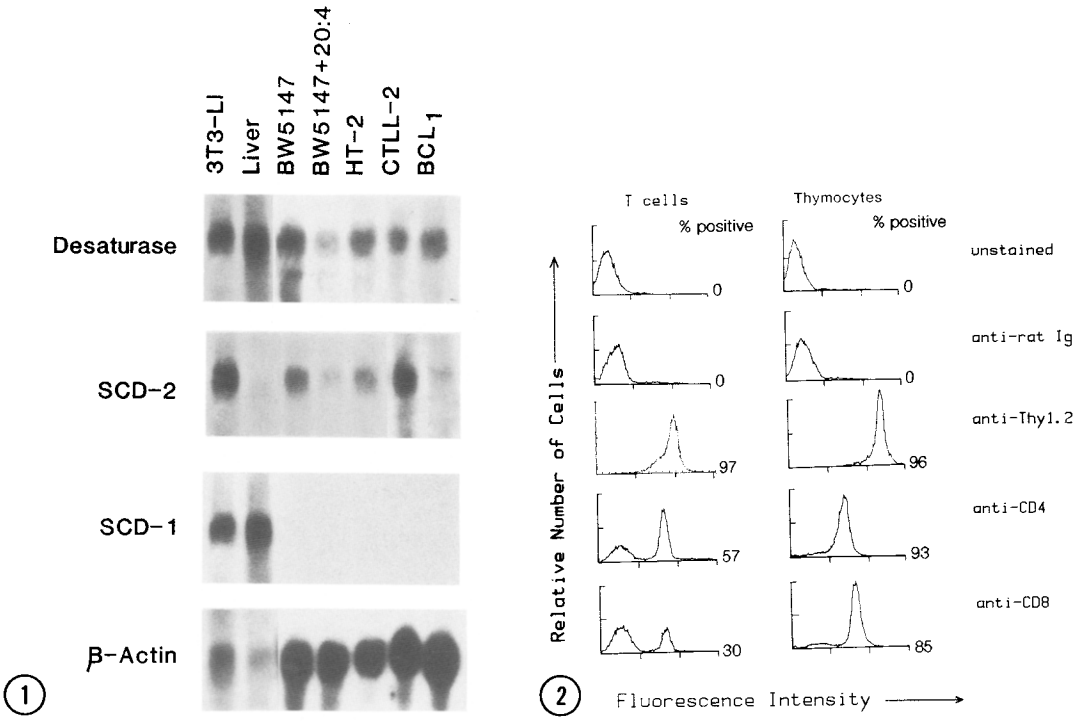

Figure 1. RNase protection assay of SCD-1 versus SCD-2 expression in BALB/c and MRL/*lpr* B and T lymphocytes and BALB/c thymocytes. Antisense RNA probes for SCD-1, SCD-2 and mouse ß-actin were synthesized using T7 or SP6 RNA polymerases, linearized plasmid templates and [α-^{32}P]UTP. The probes (80,000- 100,000 cpm) were hybridized to 5 μg RNA at 42° C in 80% formamide followed by digestion with RNases A and T_1. Protected RNA fragments were isolated by EtOH precipitation and analyzed on 6% acrylamide, 8M urea gels. Protected RNAs were localized by autoradiography.

Figure 2. CD4 and CD8 expression by BALB/c T cell and thymocyte populations. T cells and thymocytes were purified as described in Materials and Methods. Cells were subsequently stained with anti-Thy 1.2/FITC, anti-CD4/PE or anti-CD8/FITC and analyzed by flow cytometry. FITC and PE (x-axis) and relative number of cells (y-axis) are shown on log scales.

Therefore, BALB/c B and T cells were purified and subsequently assayed for the expression of SCD-2 versus SCD-1 (Fig. 1). As expected, BALB/c B cells expressed SCD-2 but not SCD-1, while BALB/c T cells expressed neither. These data indicate that the regulation of SCD-2 in lymphocytes is cell-type specific. Such specificity may be maintained through the conserved octamer motif which constitutes part of the SCD-2 (-392 to -399 upstream of the transcriptional start site) but not the SCD-1 promoter. In B cells the octamer is required for immunoglobulin gene expression (16).

The absence of stearoyl-CoA desaturase in T cells suggests a developmental down-regulation of SCD-2 within the thymus. To investigate this possibility, immature T cells were isolated based on their expression of CD4 and CD8. As shown in Fig. 2, the purified thymocytes expressed CD4 and CD8 in proportions consistent with published reports (17,18).

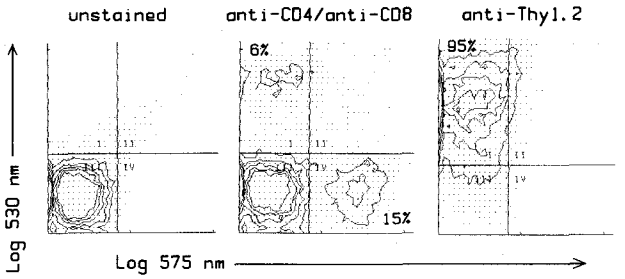

Figure 3. CD4 and CD8 expression by Thy 1.2+ MRL/*lpr* T cells. Cells were stained with anti-CD4/PE and anti-CD8/FITC or anti-Thy 1.2/FITC and analyzed by two color fluorescent flow cytometry. FITC (y-axis) and PE (x-axis) are displayed on log scales.

The isolated thymocytes were subsequently assayed for stearoyl-CoA desaturase mRNA expression (Fig. 1). In contrast to mature T cells, thymocytes expressed SCD-2 mRNA, a finding consistent with the presence of stearoyl-CoA desaturase activity in such cells (19). Current models of thymic maturation indicate that T cells arise from $CD4^-8^-$ precursors which develop into $CD4^+8^+$ cells and ultimately, into mature T lymphocytes which are either $CD4^+8^-$ or $CD4^-8^+$ (17,18). Our finding that SCD-2 is expressed in the $CD4^+8^+$ thymocytes, implies that the extinction of SCD-2 occurs late in the maturation pathway. The concept of gene down-regulation within the thymus is not novel. Phosphomyristin C, a substrate of protein kinase C, was also shown to be suppressed after the $CD4^+8^+$ stage of thymic maturation (11). The apparent down-regulation of SCD-2 predicts that maturing T cells undergo changes in membrane lipid composition and fluidity. Indeed, such was observed when the membranes from immature thymocytes were compared with those of mature thymocytes and peripheral T cells (20,21).

Additional support for a developmental down-regulation of SCD-2 is provided by studies with MRL/*lpr* mice. The *lpr* genotype contains autosomal recessive genes which predispose the mice to severe autoimmune diseases such as systemic lupus erythematosus (22). One interesting characteristic of this strain is that the peripheral T cells are primarily $CD4^-8^-$ (Fig. 3), and thus more closely resemble immature cortical thymocytes (17,18,22). As shown in Fig. 1, both B and T cells from MRL/*lpr* mice express SCD-2 mRNA. The existence of $CD4^-8^-$ T cells which express SCD-2 is consistent with aberrant T cell maturation in the autoimmune-prone MRL/*lpr* strain. It remains to be seen whether the expression of SCD-2 in MRL/*lpr* T cells predisposes the mice to the severe autoimmune disorders that are characteristic of this strain (22).

Finally, a variety of lymphoid cell lines were compared with 3T3-L1 adipocytes which express both SCD-1 and SCD-2 (5) and liver which expresses only SCD-1 (5). As seen in Fig. 4, the BCL_1 cell line resembled BALB/c B cells with respect to SCD-2 expression. However,

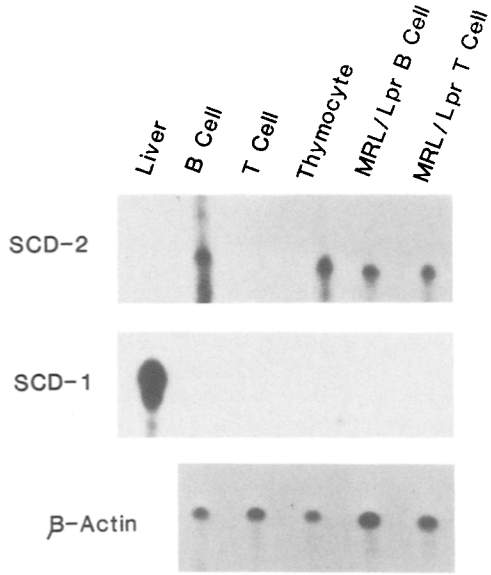

Figure 4. Northern analyses of SCD-1 versus SCD-2 expression in various lymphoid cells, BALB/c liver and 3T3-L1 adipocytes. Lymphoid cell lines were grown 10% FBS/RPMI. BW5147 cells were cultured in 1% FBS/RPMI and supplemented with 12 μM albumin-bound 20:4. Northern blots were sequentially hybridized, stripped and rehybridized with cDNA probes for rat liver stearoyl-CoA desaturase, SCD-2, SCD-1 and ß-actin.

in contrast to BALB/c T cells, the immature T cell lymphoma BW5147 expressed SCD-2, as did the cytotoxic T cell line, CTLL-2 and the helper T cell, HT-2. The expression of SCD-2 in the various T cell lines seems to be at variance with the normal situation. One possibility is that SCD-2 expression in the T cell lines reflects a selection for cells harboring a mutation which enables them to synthesize 18:1, thus permitting the rapidly proliferating cells to maintain a normal membrane fluidity. Such a mutation may be mediated by the octamer motif since some T cell lines express the B cell-specific, octamer-binding transcription factor, OCT-2 (23,24). Alternatively, SCD-2 expression in the T cell lines may be upregulated as a consequence of culture in a medium deficient in UFAs. Recent results in our laboratory have shown that stearoyl-CoA desaturase gene expression is down-regulated in the presence of 20:4 [25; P.W. Tebbey and T.M. Buttke, manuscript submitted]. The data in Fig. 4 confirms and extends our previous findings by showing that the addition of 20:4 reduces the accumulation of SCD-2 mRNA. Hence, the expression of SCD-2 in the T cell lines may result from the limited amount of 20:4 provided by the *in vitro* culture medium.

In conclusion, the data presented herein show that the regulation of SCD-2 in lymphoid cells is complex and multifaceted. Accordingly, lymphoid cell lines should provide a useful cell culture system for defining the elements which control SCD-2 gene expression.

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REFERENCES

1. Volpe, J.J. and Vagelos, P.R. (1976) Phys. Rev. 56, 339-417.
2. Jeffcoat, R. (1977) Biochem. Soc. Trans. 5, 811-818.
3. Bloomfield, D.K. and Bloch, K. (1960) J. Biol. Chem. 235, 337-344.
4. Ntambi, J.M., Buhrow, S.A., Kaestner, K.H., Christy, R.J., Sibley, E., Kelly, T.J. and Lane, M.D. (1988) J. Biol. Chem. 263, 17291-17300.
5. Kaestner, K.H., Ntambi, J.M., Kelly, T.J. and Lane M.D. (1989) J. Biol. Chem. 264, 14755-14761.
6. Pourbohloul, S., Mallett, G.S. and Buttke, T.M. (1985) Immunology 56, 659-666.
7. Pourbohloul, S. and Buttke, T.M. (1990) Int. J. Immunopharmac. 12, 799-807.
8. Buttke, T.M., Van Cleave, S., Steelman, L. and McCubrey, J.A. (1989) Proc. Natl. Acad. Sci. USA 86, 6133-6137.
9. Tebbey, P.W. and Buttke, T.M. (1990) Immunology 70, 379-384.
10. Buttke, T.M., Mallett, G.S. and Cuchens, M.A. (1983) J. Immunol. Meth. 58, 193-207.
11. Hornbeck, P., Nakabayshi, H., Fowlkes, B.J., Paul, W.E. and Kligman, D. (1989) Mol. Cell. Biol. 9, 3727-3735.
12. Melton, D.A., Krieg, P.A., Rebaghiati, M.R., Maniatis, T., Zinn, K. and Green, M.R. (1984) Nuc. Acid. Res. 12, 7035-7056.
13. Strittmatter, P., Thiede, M.A., Hackett, C.S. and Ozols, J. (1988) J. Biol. Chem. 263, 2532-2535.
14. Schriener, G.F., Flye, W., Brunt, E., Korber, K. and Lefkowith, J.B. (1988) Science 240, 1032-1033.
15. Stephens, J.M. and Pekala, P.H. (1991) J. Biol. Chem. 266, 21839-21845.
16. Wirth, T, Staudt, L. and Baltimore, D. (1987) Nature 329, 174-178.
17. Husmann, L.A., Shimonkevitz, R.P., Crispe, N.I. and Bevan, M.J., (1988) J. Immunol. 141, 736-740.
18. Egerton, M., Scollay, R. and Shortman, K. (1990) Proc. Natl. Acad. Sci. USA 87, 2579-2582.
19. Zevallos, M.G. and Farkas, T. (1989) Arch. of Biochem. and Biophys. 271, 546-552.
20. Del Buono, B.J., Williamson, P.L. and Schlegel, R.A. (1986) J. Cell. Physiol. 126, 379-388.
21. McEvoy, L., Schlegel, R.A., Williamson, P. and Del Buono, B.J. (1988) J. Leuk. Biol. 44, 337-344.
22. Cohen, P.L. and Eisenberg, R.A. (1991) Annu. Rev. Immunol. 9, 243-269.
23. Staudt, L.M., Clerc, R.G., Singh, H., LeBowitz, J.H., Sharp, P.A. and Baltimore, D. (1988) Science 241, 577-580.
24. Cockerill, P.N. and Klinken, S.P. (1990) Mol. Cell Biol. 10, 1293-1296.
25. Tebbey, P.W. and Buttke, T.M. (1992) FASEB J. 6, A1935.