Brief Definitive Report

RETINOIC ACID–INDUCED GENE EXPRESSION IN NORMAL AND LEUKEMIC MYELOID CELLS

By MICHAEL P. MURTAUGH, OLIVIA DENNISON, JOSEPH P. STEIN, AND PETER J. A. DAVIES

From the Departments of Pharmacology and Medicine, The Medical School, University of Texas Health Science Center at Houston, Houston, Texas 77225

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024

Retinoids, a group of naturally occurring lipid molecules structurally related to Vitamin A (trans-retinol), exert profound effects on the growth and differentiation of a wide variety of cells and tissues (1). Myeloid cells in particular seem to be targets of retinoid action. The differentiation of both normal and leukemic myeloid cells can be altered by exposure of the cells to retinoids, and it has been suggested that retinoids may play a physiological role in regulating the terminal maturation of these cells (2). In spite of the potential importance of retinoids as regulators of myeloid cell development, little information is available on the molecular mechanisms that are involved in this process. Recently we reported that retinoids can dramatically increase the synthesis of the enzyme tissue transglutaminase in both normal and leukemic myeloid cells (3, 4), and activation of macrophages also leads to marked induction of the enzyme (5). We believe that the induction of tissue transglutaminase is a marker of myeloid cell differentiation and a valuable tool for studying the molecular basis of retinoid action. To further our understanding of the mechanisms involved in retinoid action in myeloid cells, we have investigated the effects of all-trans-retinoic acid (retinoic acid) on the levels of tissue transglutaminase mRNA in both mouse peritoneal macrophages and human promyelocytic leukemia (HL-60) cells. We report here that in both of these cell types, retinoic acid induces a dramatic increase in the levels of tissue transglutaminase mRNA within 30 min. These studies provide experimental confirmation for the proposition that retinoids can induce acute and specific alterations in gene expression in both normal and malignant myeloid cells.

Materials and Methods

ICR strain adult male mice were obtained from Harlan Sprague-Dawley, Inc. (Houston, TX). HL-60 cells adapted to grow in serum-free media were cultured under conditions described previously (4). Retinoic acid was a gift of Hoffman-LaRoche Inc., (Nutley, NJ). CsCl, dibutyryl cyclic AMP (Bt_2cAMP), insulin, transferrin, and sodium selenite were purchased from Sigma Chemical Co. (St. Louis, MO). Delipidized mouse serum was prepared as described previously (3). Oligo-dT–cellulose was purchased from Bethesda Research Laboratories (Bethesda, MD). RPMI 1640 media was purchased from Gibco Laboratories (Grand Island, NY). A reticulocyte lysate translation kit and [^{35}S]methionine

Support for these studies was provided by grants AM27078 from the National Institutes of Health and BC-334 from the American Cancer Society. P. J. A. Davies is an Established Investigator of the American Heart Association. Address correspondence to Dr. Peter Davies, Department of Pharmacology, P. O. Box 20708, University of Texas Medical School, Houston, TX 77225.

were purchased from New England Nuclear (Boston, MA). [^{14}C]amino acids were from Becton-Dickinson Immunodiagnostics (Orangeburg, NY). The properties of the goat anti–tissue transglutaminase antibodies used in these studies have been reported previously (5).

Isolation of RNA and In Vitro Translation. Mouse resident peritoneal macrophages, isolated by peritoneal lavage and attachment to plastic tissue culture dishes (5), were cultured in RPMI 1640 media and 2% delipidized mouse serum in the presence or absence of 1 μM retinoic acid. HL-60 cells were grown in suspension culture in RPMI 1640 medium supplemented with sodium selenite (3 nM), insulin (5 μg/ml), and transferrin (5 μg/ml). Tissue transglutaminase was induced by the addition of 1 mM Bt_2cAMP and 1 μM retinoic acid.

To isolate macrophage RNA, cell monolayers were washed three times with PBS, and solubilized in the lysis buffer described by Chirgwin et al (6). Total RNA was then isolated by C_sCl gradient centrifugation (6). Polyadenylated macrophage RNA was prepared from 2×10^9 resident peritoneal macrophages (1,150 mice) incubated 6 h in medium containing 1 μM retinoic acid. Total RNA (1.6 mg) was chromatographed on oligo-dT–cellulose (7). Total RNA was isolated from HL-60 cells grown in suspension culture. Cell pellets were washed three times in PBS and lysed in 7 M guanidine HCl. DNA was removed by differential precipitation from ethanol and proteins were extracted with chloroform/isobutanol (4:1). RNA was purified by differential precipitation (6), and polyadenylated RNA was obtained by oligo-dT cellulose chromatography (7).

Total RNA and polyadenylated RNA were translated using a commercial reticulocyte lysate kit containing [^{35}S]methionine. The translation products were either solubilized directly in SDS-containing gel mix, or they were immunoprecipitated by the addition of 5 μg of affinity-purified goat anti–guinea pig tissue transglutaminase to a solution containing the translation mixture (4×10^4 cpm for macrophage translations, 10^6 cpm for HL-60 translations). Immunoprecipitated proteins were solubilized with SDS-gel mix, fractionated by SDS-gel electrophoresis on 6.5% discontinuous polyacrylamide slab gel, and identified by fluorography as described previously (5).

Results

To observe the effect of retinoic acid on tissue transglutaminase mRNA levels in mouse peritoneal macrophages, cells were cultured for 6 h in the presence or absence of 1 μM retinoic acid. Total RNA was then isolated from the cells and translated in vitro. The two bands in the control without RNA (Fig. 1, lane *1*) are due to the nonenzymatic coupling of methionine to reticulocyte proteins (8). Translation of macrophage RNA resulted in the synthesis of numerous polypeptides, most of which were equivalent in the samples obtained from either control or retinoic acid-treated cell RNA (Fig. 1, lane *2* vs. lane *3*). Three new bands, at 78, 41, and 38 kD, were prominent in the translation products of RNA from the retinoic acid–treated cells. The 78-kD band had an electrophoretic mobility very similar to purified guinea pig tissue transglutaminase and was specifically immunoprecipitated by an anti–tissue transglutaminase antibody (lanes *4* and *5*). The other two retinoic acid–induced bands, at 41 and 38 kD, were not precipitated by the anti–tissue transglutaminase antibodies. Polyadenylated RNA, purified from retinoic acid–treated macrophages and translated in vitro, also showed a prominent 78 kD-band that was immunoprecipitated by the anti–tissue transglutaminase antibodies (Fig. 1, lanes *6* and *7*).

To determine if retinoic acid also induced tissue transglutaminase mRNA in HL-60 cells, polyadenylated RNA was purified from untreated and retinoic acid–treated HL-60 cells and translated in vitro. Tissue transglutaminase was not detected in the total translation products of control cell RNA (Fig. 1, lane *8*) or its immunoprecipitate (Fig. 1, lane *9*). However, the translation products of

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024

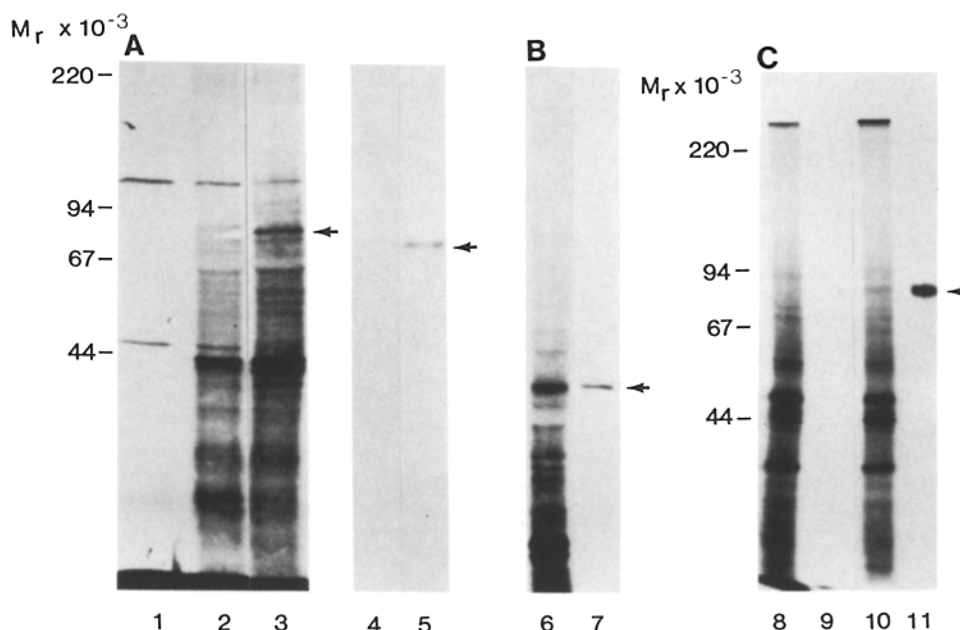

FIGURE 1. In vitro translation of RNA from retinoic acid–treated macrophages and HL-60 cells. (*A*) Mouse peritoneal macrophages were cultured for 6 h in RPMI medium plus 2% delipidized serum alone (control) or with 1 μM retinoic acid (RA-treated). RNA was isolated from the cells, translated using a reticulocyte lysate and [^{35}S]methionine. The total translation products from a blank without RNA (lane *1*), control cell RNA (lane *2*), and RA-treated cell RNA (lane *3*) were solubilized in SDS-gel mix and fractionated on a 6.5% polyacrylamide slab gel. Translation products from control cell and RA-treated cell RNA were also immunoprecipitated with an antibody to tissue transglutaminase (lanes *4* and *5* respectively). (*B*) Polyadenylated RNA, purified from RA-treated macrophages, was translated and either fractionated on a 6.5% polyacrylamide slab gel (lane *6*) or immunoprecipitated with an anti–tissue transglutaminase antibody (lane *7*). (*C*) HL-60 cells were cultured for 18 h in media containing 1 mM Bt_2cAMP (control) or 1 μM retinoic acid and 1 mM Bt_2cAMP (RA-treated). Polyadenylated RNA from control and RA-treated cells was translated and then either electrophoresed on a 10% SDS-acrylamide gel (lanes *8* and *10*) or immunoprecipitated with anti–tissue transglutaminase antibody (lanes *9* and *11*). *Arrows*, mobility of purified tissue transglutaminase.

retinoic acid–treated cell RNA revealed a band at 82 kD (lane *10*, *arrow*) that was immunoprecipitated by anti–tissue transglutaminase IgG (Fig. 1, lane *11*).

To follow the time course for the induction of tissue transglutaminase mRNA, resident peritoneal macrophages were exposed to 1 μM retinoic acid for various lengths of time; total RNA was isolated, translated in vitro, and the tissue transglutaminase immunoprecipitated (Fig. 2*A*). In untreated cells and cells treated for up to 30 min with retinoic acid (Fig. 2, lanes *1* and *2*, respectively), the levels of tissue transglutaminase mRNA were very low. Cells treated for >30 min, however, showed increasing accumulation of tissue transglutaminase mRNA as indicated by an increasingly intense band of tissue transglutaminase in the immunoprecipitates (lanes *3*–*6*). Quantitation of the transglutaminase band by densitometry indicated a linear increase in the levels of transglutaminase mRNA that started ~30 min after the addition of the retinoid (Fig. 2*B*).

To determine if there was posttranslational processing of tissue transglutaminase in myeloid cells, we compared the electrophoretic mobility of tissue transglutaminase synthesized in vitro with that immunoprecipitated from metabolically

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024

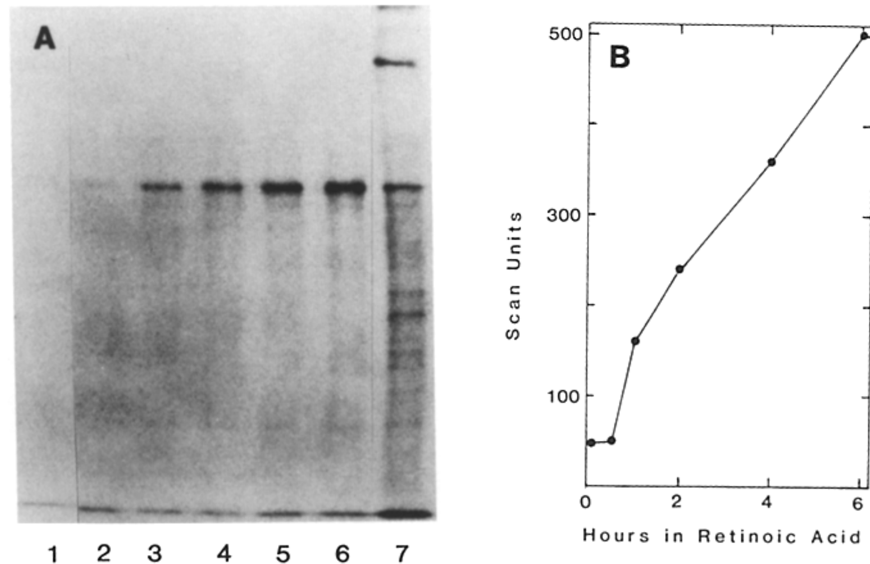

FIGURE 2. Time course for retinoic acid-induced expression of tissue transglutaminase mRNA in cultured mouse peritoneal macrophages. (*A*) Mouse peritoneal macrophages were cultured in medium containing 1 μM retinoic acid for 0 (lane *1*), 0.5 (lane *2*), 1 (lane *3*), 2 (lane *4*), 4 (lane *5*) and 6 h (lane *6*). Total RNA was isolated, translated, and immunoprecipitated with an anti-transglutaminase antibody and then fractionated on a 10% polyacrylamide slab gel. Macrophages cultured with retinoic acid for 5 h also were pulse-labeled with [^{35}S] cysteine and a cell lysate prepared in SDS was electrophoresed in lane 7. (*B*) The densities of the tissue transglutaminase bands in lanes *1–6* were quantified by laser densitometry.

labeled cells (Fig. 3). Immunoprecipitates from metabolically labeled HL-60 cells showed a major band of tissue transglutaminase at 82 kD (Fig. 3, *arrow*). Tissue transglutaminase synthesized from HL-60 cell RNA had the same electrophoretic mobility (lane *2*). A mixture of equal amounts of the metabolically labeled protein and the protein synthesized in vitro gave a single band (lane *3*). Similar results were obtained with mouse macrophage transglutaminase synthesized in vitro and in vivo (Fig. 2*A*, lanes *6* and *7*). These results show that neither human nor mouse tissue transglutaminase are subjected to extensive posttranslational processing.

Discussion

It is often assumed that retinoids, like steroid hormones, have their primary effect at the level of the genome, regulating the expression of specific proteins (9). However, at present there is little experimental evidence that retinoids directly regulate gene expression. Retinoids alter the expression of a variety of cellular proteins in a number of different cultured cell lines (1, 10), but these effects are generally the result of chronic exposure to retinoids. Such chronic effects may reflect secondary consequences of retinoid-induced differentiation rather than a primary effect of the retinoid on gene expression itself. Our results provide more direct evidence that, at least in myeloid cells, retinoids can induce acute alterations in gene expression. Increased levels of tissue transglutaminase mRNA were detected 30 min or more after the addition of retinoic acid to cultured macrophages. Given the relatively insensitive assay techniques we have

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024

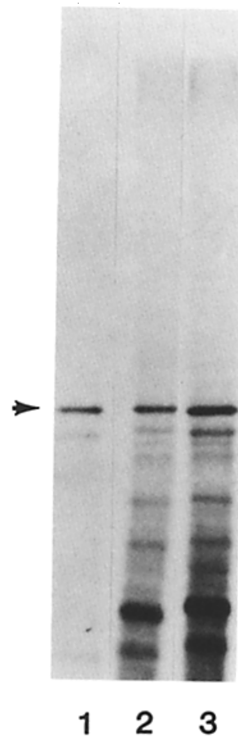

FIGURE 3. Comparison of HL-60 cell tissue transglutaminase synthesized in vitro and in intact cells. HL-60 cells were incubated for 18 h in medium containing 1 μM retinoic acid and 1 mM Bt_2cAMP, and either [^{14}C]amino acids or RNA was isolated from the cells and translated in vitro. Tissue transglutaminase was immunoprecipitated from the metabolically labeled cell extract (lane *1*, *arrow*) or the in vitro translation reaction mix (lane *2*). Lane *3* shows a mixture of equal amounts of tissue transglutaminase from metabolically labeled cells and the in vitro translation reaction.

used and the time required for mature mRNA molecules to accumulate in the cytoplasm, our results suggest that altered gene expression follows very rapidly the addition of retinoids to macrophages.

The extent of transglutaminase induction in macrophages and HL-60 cells is remarkable. Our previous studies have shown that fresh serum or retinoids cause the enzyme to accumulate up to 1% of total cellular protein in 24 h (3–5). This enormous induction appears to be due to a very large induction of tissue transglutaminase mRNA. In unstimulated macrophages, the level of tissue transglutaminase mRNA is low. In retinoid-treated cells, on the other hand, the enzyme can easily be identified in the translation products without the need for immunoprecipitation, suggesting that tissue transglutaminase mRNA has become one of the most abundant mRNA in the cell.

It has long been recognized that a nutritional deficiency in vitamin A is associated with a significant impairment of immune function (11). Although the cellular basis for this dysfunction is not known, the high incidence of generalized infections and decreased efficacy of tumor cell killing in retinoid-deficient animals suggests that macrophage function may be suppressed (11). Our finding that physiological levels of retinoids can have profound effects on gene expression in

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024

macrophages supports the idea that these metabolites of vitamin A may be important regulators of immune function.

Summary

Retinoic acid has been shown to induce large accumulations of tissue transglutaminase in cultured myeloid cells. Addition of retinoic acid to mouse resident peritoneal macrophages increased the level of tissue transglutaminase mRNA within 30–60 min. Retinoic acid also increased tissue transglutaminase mRNA levels in human promyelocytic leukemia (HL-60) cells. These studies show that retinoic acid can induce acute alterations in specific gene expression in both normal and leukemic myeloid cells.

Received for publication 24 July 1985.

References

1. Roberts, A. B., and M. B. Sporn. 1984. Cellular biology and biochemistry of the retinoids. In The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and DeW. S. Goodman, editors. Academic Press Inc., Orlando, FL. 210–276.
2. Breitman, T. R., S. E. Selonick, and S. J. Collins. 1980. Induction of the differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci. USA.* 77:2936.
3. Moore, W. T., M. P. Murtaugh, and P. J. A. Davies. 1984. Retinoic acid induced expression of tissue transglutaminase in mouse peritoneal macrophages. *J. Biol. Chem.* 259:12794.
4. Davies, P. J. A., M. P. Murtaugh, W. T. Moore, G. S. Johnson, and D. Lucas. 1985. Retinoic-acid induced expression of tissue transglutaminase in human promyelocytic leukemia (HL-60) cells. *J. Biol. Chem.* 260:5166.
5. Murtaugh, M. P., K. Mehta, J. Johnson, M. Myers, R. J. Juliano, and P. J. A. Davies. 1983. Induction of tissue transglutaminase in mouse peritoneal macrophages. *J. Biol. Chem.* 258:11074.
6. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294.
7. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA* 69:1408.
8. Jackson, R. J., and T. Hunt. 1983. Preparation and use of nuclease-treated rabbit reticulocyte lysates for the translation of eukaryotic messenger RNA. *Methods Enzymol.* 96:50.
9. Sporn, M. B., and A. B. Roberts. 1983. Role of retinoids in differentiation and carcinogenesis. *Cancer Res.* 43:3034.
10. Omori, M., and F. Chytil. 1982. Mechanism of vitamin A action. *J. Biol. Chem.* 257:14370.
11. Dennert, G. 1984. Retinoids and the immune system: immunostimulation by vitamin A. *In* The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and DeW. S. Goodman, editors. Academic Press Inc., Orlando, FL. 373–388.

Downloaded from http://rupress.org/jem/article-pdf/163/5/1325/1666777/1325.pdf by guest on 24 April 2024