

# Methotrexate induces differentiation of human keratinocytes

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**ABSTRACT** Terminal differentiation is a key element in the maintenance of tissue homeostasis in the epidermis. We show here that methotrexate (MTX) induces differentiation of human epidermal keratinocytes *in vitro*. MTX inhibits proliferation of keratinocytes and also induces several markers of differentiation: a change in cell morphology, a marked increase in cell size, an increase in the proportion of cells that express involucrin, and an increase in the amount of cornified envelope protein. These effects of MTX are dose- and exposure-time-dependent and become irreversible after 24 hr, approximately one population doubling time. These effects of MTX cannot be attributed to cytotoxicity since keratinocytes not only remain viable but also actively synthesize proteins. MTX causes reproducible changes in the SDS/PAGE profiles of newly synthesized proteins and, in particular, increases the amount of involucrin synthesis. Thymidine completely prevents these effects of MTX, suggesting that they are caused by a depletion of thymine deoxyribonucleotides. The effect of MTX on keratinocytes may provide a model for studying the relationship between deoxyribonucleotide metabolism and differentiation in normal cells. In addition, the ability of MTX to induce differentiation in keratinocytes suggests a mechanism to explain its therapeutic action in psoriasis.

The normal human epidermis is a continuously renewing epithelium in which proliferation of keratinocytes is perfectly matched by loss of keratinocytes through differentiation and ultimate desquamation (1). Epidermal hyperplasia occurs when either the rate of individual cell proliferation or the growth fraction increases (2, 3). It has been proposed that one way to control hyperplasia or neoplasia is to induce differentiation, thereby limiting or reducing the growth fraction (4–6).

Differentiation of keratinocytes in the epidermis is marked by a temporally and spatially well-defined sequence of morphological and biochemical changes (1, 7). Differentiation of keratinocytes in culture is marked by many of the changes that occur *in vivo*: an increase in cell size (8); acquisition of a cornified cell envelope (9); expression of structural proteins (7, 10–12), envelope precursors such as involucrin (8), and the transglutaminase (10) that cross-links the envelope proteins.

Methotrexate (MTX) is an antifolate that was designed to inhibit cell proliferation by blocking *de novo* synthesis of nucleotide precursors of nucleic acid synthesis (13). Some cells can be "rescued" after a long exposure to cytostatic concentrations of MTX suggesting that in these systems the effects of MTX are largely reversible (14). In other cells, MTX can cause irreversible physiologic changes such as DNA damage (15) and, in some cases, induction of differentiation (16–18).

MTX is an important therapy for psoriasis, a benign hyperproliferative disease of epidermal keratinocytes. The accepted rationale for its use is that it causes a temporary

reversible inhibition of DNA synthesis and cell proliferation (19). But that explanation for its efficacy has never been entirely convincing, leading to suggestions that it acts indirectly, for example, on the immune system (19).

We recently observed, while studying the antiproliferative effects of MTX (20), that it also had a pronounced effect on keratinocyte morphology and size. Since similar morphologic changes often accompany keratinocyte differentiation *in vitro*, we tested the hypothesis that MTX could induce differentiation and bring about an irreversible arrest of keratinocyte growth.

## METHODS

**Cell Culture.** Cultures of human neonatal foreskin keratinocytes were grown and maintained as described (20, 21). Second- or third-passage keratinocytes were seeded at  $0.5\text{--}1 \times 10^3$  cells per  $\text{cm}^2$  in complete MCDB 153 medium. Complete MCDB 153 medium contains 0.1 mM  $\text{Ca}^{2+}$ , bovine pituitary extract (50  $\mu\text{g}/\text{ml}$ ), epidermal growth factor (10 ng/ml), insulin (5  $\mu\text{g}/\text{ml}$ ), hydrocortisone (0.5  $\mu\text{g}/\text{ml}$ ), and additional amino acids; this medium is formulated with 3  $\mu\text{M}$  thymidine, 180  $\mu\text{M}$  adenine, and 1.8  $\mu\text{M}$  folic acid. Cultures were fed the following day and used for experiments 2–3 days after seeding.

**Cell Proliferation, Size, and Viability.** Keratinocytes in 35-mm culture dishes were fed with 2 ml of complete MCDB 153 medium, formulated without thymidine, in the presence or absence of various concentrations of MTX. In some experiments, 5  $\mu\text{M}$  thymidine was added and, in others, the concentration of  $\text{Ca}^{2+}$  was increased to 2 mM by addition of  $\text{CaCl}_2$ . In experiments conducted for 5–6 days, cultures were refed with experimental medium on day 3. At various times, cells were removed from the dish with trypsin and counted in a Coulter counter, model B. Proliferation was measured as an increase in number of cells per dish.

The distribution of cells by volume was determined for trypsinized cells using a Coulter counter, model ZM, equipped with a Channelyzer, model 256. Median cell volume was measured for populations of  $>12,000$  cells. Particles  $<600 \mu\text{m}^3$  were disregarded.

The viability of cells was determined by staining with trypan blue or neutral red (Clonetics, San Diego).

**Immunocytochemical Analysis for Involucrin.** Involucrin-positive cells in culture dishes were identified by modification of a previously described method (21). Briefly, cells in culture dishes were washed with phosphate-buffered saline (PBS) and permeabilized with methanol containing 0.3%  $\text{H}_2\text{O}_2$ . Nonspecific protein binding was blocked with normal swine serum and then cells were exposed to rabbit anti-human involucrin antibody (Biomedical Technologies, Stoughton, MA) followed by swine anti-rabbit IgG (DAKO, Carpinteria, CA) with intermediate washes using PBS. Finally, cells were exposed to rabbit horseradish peroxidase-anti-horseradish peroxidase complex (DAKO). Peroxidase activity was detected with

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Abbreviation: MTX, methotrexate.

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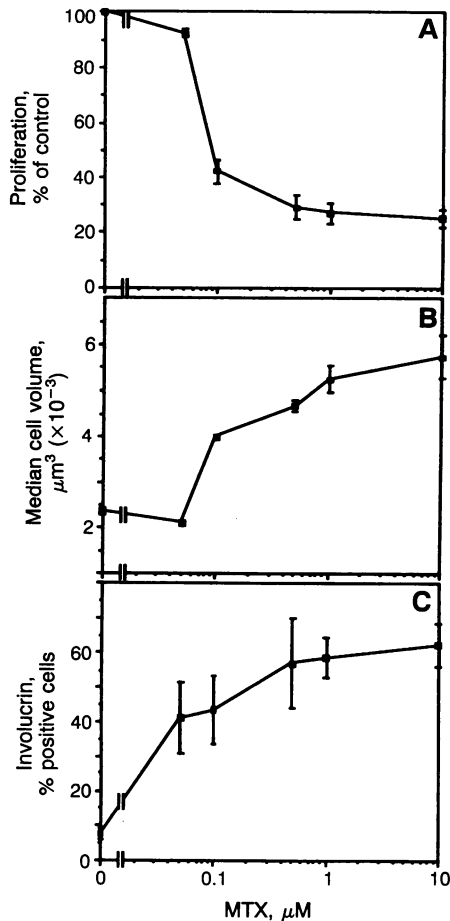


FIG. 1. Effect of MTX on keratinocyte proliferation, volume, and expression of involucrin. Preconfluent cells were treated for 72 hr in thymidine-free medium. (A) Cell proliferation is expressed as a percent of growth in control drug-free cultures. Control cultures grew from  $1.5 \times 10^5$  to  $7.5 \times 10^5$  cells per dish in 72 hr. (B) The median cell volume of cells was measured electronically. (C) Involucrin expression is shown as a percent of total cells that stain with anti-involucrin antibody. In control cultures, the number of involucrin-positive cells initially was  $1.3 \times 10^4$  cells per dish and, at 72 hr, was  $5.5 \times 10^4$  cells per dish; in MTX-treated cells, after 72 hr, the number of involucrin-positive cells was  $1.0 \times 10^5$  cells per dish. Data (mean  $\pm$  SEM) are from three experiments with duplicate determinations.

3,3'-diaminobenzidine. Cells processed as above, but without exposure to the involucrin antibody, showed no staining.

**Protein Synthesis, SDS/PAGE, and Fluorography.** Cells were exposed to [ $^3$ H]leucine (20  $\mu$ Ci/ml; 1 Ci = 37 GBq) for 6 hr, washed with PBS, and removed from the dish with trypsin, and radioactivity in a sample was measured. After centrifugation, the cell pellet was suspended in 10% (wt/vol) SDS and radioactivity was measured by scintillation counting.

In separate experiments, radioactively labeled proteins were solubilized and boiled in 40 mM Tris-HCl (pH 8.8) containing 4% SDS, 20 mM dithiothreitol, 5 mM EDTA, 15% (wt/vol) sucrose, and 0.02% bromophenol blue. Proteins were separated by SDS/PAGE on a 4–15% polyacrylamide gradient gel using a discontinuous buffer system (22). Molecular mass markers and purified involucrin (BTI) were analyzed concurrently. Gels were stained with Coomassie brilliant blue R250, impregnated with fluor (Amplify, Amersham), dried, and exposed to Kodak X-Omat/AR film for 4 days at  $-70^\circ\text{C}$  before development.

**Analysis of Cornified Envelope Protein.** Cross-linked cornified envelope protein was determined by the method of Hough-Monroe and Milstone (23). Briefly, cells were re-

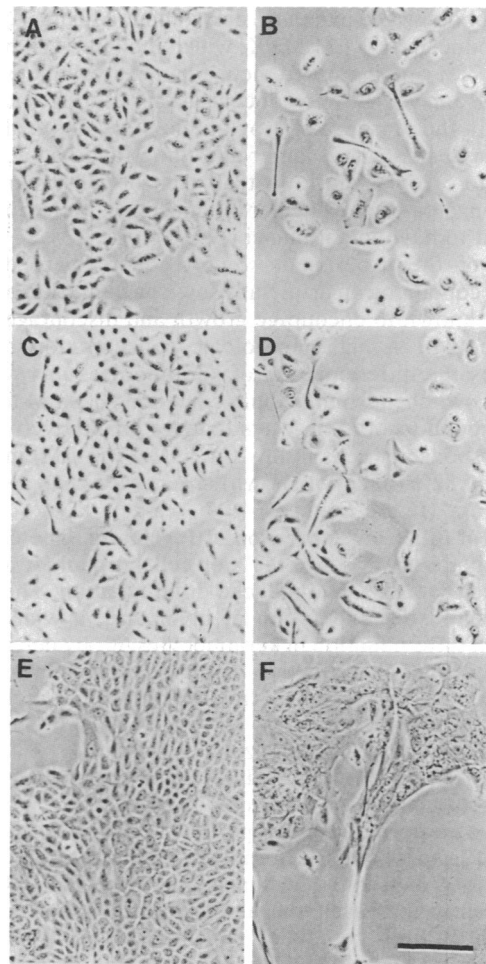


FIG. 2. Effect of MTX on morphology of keratinocytes. Cells were treated for 72 hr in the presence or absence of 1  $\mu$ M MTX and 5  $\mu$ M thymidine was added at indicated times. The effect of MTX is shown in medium containing low  $\text{Ca}^{2+}$  (0.1 mM). (A) Control. (B) MTX-treated cells. (C) MTX-treated cells with thymidine added simultaneously with MTX. (D) MTX-treated cells with thymidine added 24 hr after MTX. The effect of MTX is shown in medium containing high  $\text{Ca}^{2+}$  (2.0 mM). (E) Control. (F) MTX-treated cells. Cultures were photographed using phase-contrast microscopy. (Bar = 200  $\mu$ m.)

moved from the culture dish with trypsin and counted. After centrifugation, the cell pellet was resuspended in 1% SDS/0.2% 2-mercaptoethanol to give  $1 \times 10^5$  whole cells per ml and the cell suspension was boiled for 5 min. The insoluble cross-linked protein was separated from soluble proteins by filtration on a regenerated cellulose paper (Schleicher & Schuell) in a 96-well filtration manifold. Soluble protein was washed through with 1% SDS/0.2% 2-mercaptoethanol. The filter was then fixed with 7.5% (wt/vol) trichloroacetic acid, washed, and stained with Coomassie blue G250. Unbound stain was removed with 7% (vol/vol) acetic acid. Intensity of the stained dot is directly proportional to the number of cornified envelopes.

**Chemicals and Medium.** MTX and thymidine were purchased from Sigma. Basal MCDB 153 medium formulated without thymidine was purchased from Clonetics. L-[3,4,5- $^3\text{H}$ (N)]Leucine was purchased from NEN.

## RESULTS

**Effect of MTX on Proliferation, Morphology, and Cell Size.** We have demonstrated (20) that MTX inhibited the proliferation of keratinocytes in thymidine-free complete MCDB 153 medium. Along with its effects on proliferation, MTX altered

cell morphology and increased cell volume. Maximal changes were produced by 1  $\mu\text{M}$  MTX, which inhibited cell proliferation by 75% and increased the median cell volume by 225% (Fig. 1 A and B). Lower concentrations of drug had lesser effects. In the presence of growth-inhibitory doses of MTX, keratinocytes appeared larger and flatter and had a smaller nuclear-to-cytoplasmic ratio than control cells; some cells were elongated or polyangular but most cells were round; all appeared to have intact nuclei (Fig. 2 A–D).

Most of the experiments reported here were done in medium containing an artificially low concentration of  $\text{Ca}^{2+}$ , which is known to encourage growth and discourage differentiation (24). A high physiologic concentration of  $\text{Ca}^{2+}$  facilitates the differentiation of keratinocytes *in vitro* (24). Cultures switched from medium containing 0.1 mM to 2 mM  $\text{Ca}^{2+}$  respond to MTX in ways that are qualitatively similar to the response of cells in low  $\text{Ca}^{2+}$ . MTX (1  $\mu\text{M}$ ) inhibited growth by 82% (data not shown) and caused changes in cell morphology (Fig. 2 E and F).

**Effect of MTX on Expression of Involucrin.** The effect of growth-inhibitory doses of MTX on cell morphology and size suggested that MTX had induced differentiation. Therefore, we measured the expression of involucrin, a widely used marker of differentiation in keratinocytes (8), by determining the proportion of cells that expressed involucrin. MTX increased the proportion of involucrin-positive cells in a dose-dependent manner that correlated with inhibition of growth and increase in cell size (Fig. 1C).

**Effect of MTX on Cornified Envelope Protein.** One well-recognized marker of terminal differentiation in keratinocytes is the cornified envelope (9). The amount of cornified envelope protein was determined after culturing keratinocytes for 6 days in the presence or absence of 1  $\mu\text{M}$  MTX. MTX clearly increased the amount of cornified envelope protein in cultures containing either 0.1 mM or 2 mM  $\text{Ca}^{2+}$  (Fig. 3). In addition, MTX caused a severalfold increase in the proportion of cells that had cornified envelopes visualized by phase-contrast microscopy (data not shown).

**Effects of MTX on Cell Viability and Protein Synthesis.** Keratinocytes treated with 1  $\mu\text{M}$  MTX for 3 days remained viable; cells excluded trypan blue stain and stained with neutral red to the same extent as untreated keratinocytes (data not shown). Cells treated with MTX for 5 days, either in medium containing 0.1 mM or 2 mM  $\text{Ca}^{2+}$ , incorporated 2–2.3 times more radioactive leucine into proteins than untreated cells (Table 1). This increase in protein synthesis is commensurate with the increase in cell volume.

**SDS/PAGE Analysis of Proteins.** To determine whether different proteins were made in MTX-treated versus control

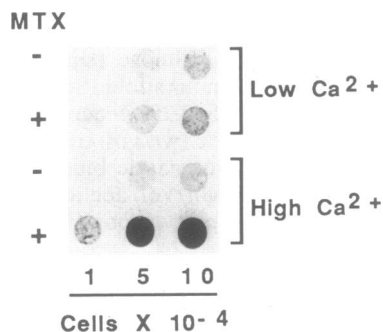


FIG. 3. Effect of MTX on cornified envelope protein. Keratinocytes were grown in medium containing either 0.1 mM or 2 mM  $\text{Ca}^{2+}$  in the presence or absence of 1  $\mu\text{M}$  MTX. On day 6, cells were removed from the culture dish and cross-linked envelope protein was prepared from equal numbers of whole cells and analyzed. Intensity of the Coomassie blue stain is proportional to the amount of envelope protein.

Table 1. Incorporation of [ $^3\text{H}$ ]leucine into proteins of keratinocytes *in vitro*

MTX, $\mu\text{M}$	$\text{Ca}^{2+}$ , mM	[ $^3\text{H}$ ]Leucine incorporation	
		cpm per $10^3$ cells	% of control
0	0.1	730 $\pm$ 70	100
1	0.1	1500 $\pm$ 110	210
0	2	900 $\pm$ 70	100
1	2	2100 $\pm$ 140	240

Keratinocytes were grown in thymidine-free medium in the presence or absence of MTX. On day 5, cultures were exposed to [ $^3\text{H}$ ]leucine for 6 hr. Cells were counted and radioactivity incorporated into protein was determined by scintillation counting.

cells, keratinocyte proteins extracted after a 6-hr pulse with [ $^3\text{H}$ ]leucine were separated by SDS/PAGE. The pattern of newly synthesized proteins was distinctly different in MTX-treated cells compared to control cells (Fig. 4). Of particular interest was the relative increase per cell in synthesis of a protein that comigrated with purified involucrin. In addition to quantitative changes in these several bands, MTX treatment appeared to induce synthesis of several proteins and suppress the synthesis of others.

**Time Course for Effects of MTX on Cell Growth, Size, and Expression of Involucrin.** Cells were treated with 1  $\mu\text{M}$  MTX in thymidine-free complete MCDB 153 medium (0.1 mM  $\text{Ca}^{2+}$ ) and harvested at various times. MTX had small effects on cell growth or other parameters at 24 hr but between 24 and 48 hr cells treated with MTX had stopped proliferating and showed a definite change in morphology and size. By 72 hr, MTX produced marked changes in cell number, cell volume, and expression of involucrin compared to untreated control cells (Fig. 5).

**Effect of Thymidine on Action of MTX.** Thymidine has been shown to prevent the growth-inhibitory activity of MTX in keratinocytes (20). To monitor the effects of thymidine on both growth and differentiation, thymidine was added to

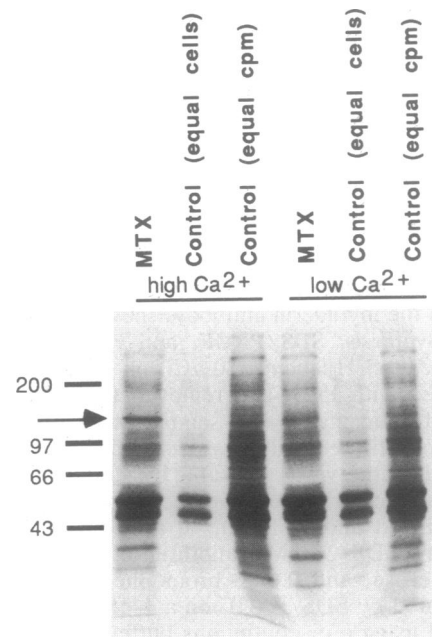


FIG. 4. Fluorogram of keratinocyte proteins separated by SDS/PAGE. Cells were grown in the presence or absence of 1  $\mu\text{M}$  MTX for 5 days and exposed to [ $^3\text{H}$ ]leucine for the last 6 hr. Radioactive proteins from MTX-treated cells were compared to control cells based either on an equal number of cells ( $9.1 \times 10^4$  cells) or an equal amount of radioactivity ( $4.7 \times 10^4$  cpm). Bars denote molecular mass standards (kDa) and the arrow denotes the migration of purified involucrin stained with Coomassie blue in an adjacent lane.

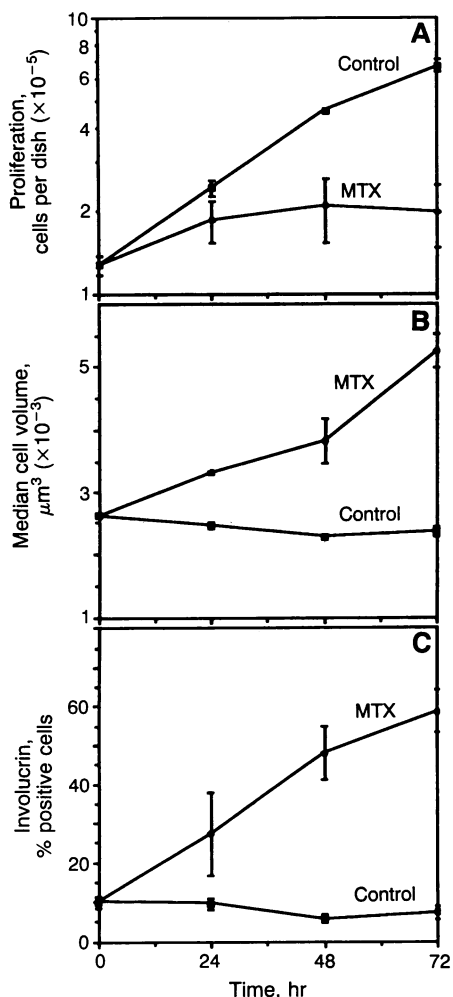


FIG. 5. Time course for effects of MTX on keratinocytes. Pre-confluent cells were treated with 1  $\mu$ M MTX in medium containing 0.1 mM  $\text{Ca}^{2+}$ . (A) Number of cells per dish. (B) Median cell volume. (C) Involucrin-positive cells expressed as a percent of total cells. Data (mean  $\pm$  SEM) are from three experiments with duplicate determinations. Data from control drug-free cultures and MTX-treated cultures are indicated.

keratinocytes at various times after initiating treatment with 1  $\mu$ M MTX. In the absence of thymidine, MTX inhibited growth and induced differentiation. Since the experimental medium contained adenine and folic acid, the immediate effects of MTX can be ascribed to its action on thymine deoxyribonucleotides. When thymidine was added to cells at the same time as MTX, the effects of MTX on growth and differentiation were prevented; in every way, these cultures appeared comparable to MTX-free control cultures (Figs. 2 and 6). Addition of thymidine 6 hr after the addition of MTX also prevented the effects of MTX. By contrast, addition of thymidine 24 hr after the addition of MTX failed to prevent the effects of MTX; growth was inhibited and percent of involucrin-positive cells increased to the same extent as in cultures receiving MTX alone.

## DISCUSSION

The results presented herein demonstrate that MTX not only inhibited the proliferation of keratinocytes *in vitro* but also induced their differentiation. While growth arrest may be a necessary consequence of keratinocyte differentiation (25), growth arrest does not necessarily result in the onset of differentiation (26–28). For example, ethionine addition or

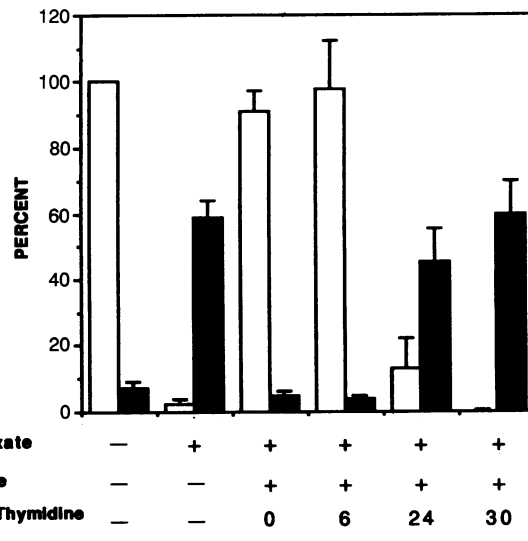


FIG. 6. Effect of thymidine on the action of MTX in keratinocytes. Cells were grown in the presence (+) or absence (-) of MTX and thymidine. Thymidine was added at the times (hr) indicated. Proliferation, shown as percent of growth in control cultures (open bars), and involucrin, shown as percent of cells that express involucrin (solid bars), were determined at 72 hr.

isoleucine starvation causes a reversible growth arrest in the  $G_1$  phase of the cell cycle that does not lead to keratinocyte differentiation (26, 27).

The conclusion that MTX induces terminal differentiation of keratinocytes is based on morphological and biochemical criteria. The first clue that MTX might be inducing differentiation was the observation of an increase in cell size and decrease in nuclear-to-cytoplasmic ratio. Although many inhibitors of DNA synthesis routinely cause an increase in cell volume that can be attributed solely to continuing protein synthesis (29), the relationship between differentiation and keratinocyte volume is well-established (8).

Nonspecific toxicity cannot explain the changes caused by MTX insofar as treated cells had grossly normal membrane function (assessed by appropriate dye exclusion and uptake) and synthesized protein 2.3 times faster than control cells. This latter number compares favorably with the 2.5-fold increase in protein synthesis found in differentiated keratinocytes that had been separated from proliferating keratinocytes by size (30).

Study of more specific biochemical markers confirmed that MTX was indeed inducing differentiation. Acquisition of a cornified cell envelope and expression of involucrin are widely used markers of keratinocyte differentiation (8, 9). The cornified cell envelope, generally considered a late marker of differentiation, consists of covalently cross-linked cell-membrane-associated proteins (10). Formation of the cornified envelope requires synthesis of specific precursors, such as involucrin, as well as synthesis and activation of the cross-linking enzyme (10). MTX increased the proportion of cells that expressed involucrin and also increased the amount of newly synthesized involucrin and total envelope protein per cell. Thus these data strongly support the contention that MTX induces keratinocyte differentiation.

The biochemical actions of MTX are well-known, thus allowing us to postulate a mechanism by which MTX induces differentiation in keratinocytes. MTX initially blocks dihydrofolate reductase, thereby preventing *de novo* synthesis of purine and pyrimidine nucleotides (13). Since our test medium contains adenine but lacks thymidine, the actions of MTX should be attributable entirely to an inhibition of *de novo* synthesis of thymine deoxyribonucleotides. Prevention

of all the effects of MTX on keratinocytes by the addition of thymidine to the culture medium provides strong evidence that MTX is acting by depleting the dTTP pool. The observation that there is a 24-hr window during which induction of differentiation by MTX can be prevented by thymidine suggests that the effect may occur at a specific point in the cell cycle. MTX blocks DNA synthesis and arrests cell growth at the G<sub>1</sub>-S interphase (14); perhaps this is also the point in the cell cycle from which differentiation is initiated by MTX.

There is precedent for irreversible disruption of normal cell physiology by an imbalance in nucleotide pools (31). Many agents that deplete dTTP pools can cause mutations due to DNA damage (31) and MTX is known to cause DNA strand breaks (15). There is also precedent for the ability of MTX to induce differentiation. In two other test systems, BeWo cells, a choriocarcinoma cell line (17), and HL-60, a promyelocytic leukemia cell line (16, 18), MTX induced differentiation that was attributed to changes in the thymine deoxyribonucleotide pools. Although reduction of dTTP pools can be caused by drugs, such as MTX, depletion of dTTP might conceivably be a physiologic signal for keratinocytes to withdraw from the cell cycle and initiate a program of differentiation. Intracellular thymine deoxyribonucleotide pools are tightly regulated (32) and we recently demonstrated a correlation between onset of differentiation in keratinocytes and loss of the capacity to synthesize dTTP from extracellular thymidine (21).

The therapeutic action of methotrexate in psoriasis, and perhaps in certain neoplastic diseases, needs reevaluation in light of the results presented here. Psoriasis is a benign hyperproliferative disease of the epidermis in which the cell cycle time is many times faster than normal and the growth fraction is nearly twice normal (33). The hypothesis that MTX is acting irreversibly in psoriasis by inducing terminal differentiation is consistent with several clinical observations. (i) Weekly pulse therapy with MTX controls hyperplasia despite the fact that inhibition of DNA synthesis lasts only 16–24 hr (34). (ii) The appearance of “MTX-damaged cells” in plaques of psoriasis (34, 35) had been attributed to cell death. (iii) Curiously, MTX resistance has not been reported in chronically treated psoriatic keratinocytes (19). These observations may be explained if MTX caused an induction of differentiation that resulted in irreversible withdrawal of cells from the proliferative compartment.

The investigations reported here demonstrate that MTX can induce differentiation in a normal cell. By inference, an inadequate supply of intracellular dTTP is the biochemical change responsible for that result. Consequently, the regulation of thymine deoxyribonucleotide pools—both in normal keratinocyte physiology and as a target for pharmacologically induced differentiation—merits further study.

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1. Milstone, L. M. (1983) *J. Invest. Dermatol.* **81**, 69S–74S.

2. Gelfant, S. (1976) *Br. J. Dermatol.* **95**, 577–590.
3. Weinstein, G. D., McCullough, J. L. & Ross, P. A. (1985) *J. Invest. Dermatol.* **85**, 579–583.
4. Reiss, M., Gamba-Vitalo, C. & Sartorelli, A. C. (1986) *Cancer Treat. Rep.* **70**, 201–218.
5. Scott, R. E., Edens, M., Estervig, D. N., Filipak, M., Hoerl, B. J., Hsu, B. M., Maercklein, P. B., Minoo, P., Tzen, C.-Y., Wilke, M. R. & Zschunke, M. A. (1988) in *The Status of Differentiation Therapy of Cancer*, eds. Waxman, S., Rossi, G. B. & Takaku, F. (Serono Symposia Publications, Raven, NY), Vol. 45, pp. 3–16.
6. Francis, G. E. & Pinsky, C. M. (1988) in *The Status of Differentiation Therapy of Cancer*, eds. Waxman, S., Rossi, G. B. & Takaku, F. (Serono Symposia Publications, Raven, NY), Vol. 45, pp. 331–347.
7. Fuchs, E. (1990) *J. Cell Biol.* **111**, 2807–2814.
8. Watt, F. A. & Green, H. (1981) *J. Cell Biol.* **90**, 738–742.
9. Green, H. (1977) *Cell* **11**, 405–416.
10. Hohl, D. (1990) *Dermatologica* **180**, 201–211.
11. Dale, B. A., Gown, A. M., Fleckman, P., Kimball, J. R. & Resing, K. A. (1987) *J. Invest. Dermatol.* **88**, 306–313.
12. Yuspa, S. H., Kilkenny, A. E., Steinert, P. M. & Roop, D. R. (1989) *J. Cell Biol.* **109**, 1207–1217.
13. Werkheiser, W. C. (1963) *Cancer Res.* **23**, 1277–1285.
14. Taylor, I. W. & Tattersall, M. H. N. (1981) *Cancer Res.* **41**, 1549–1558.
15. Jackson, D. A., Pearson, C. K., Fraser, D. C., Prise, K. M. & Wong, S. Y. (1989) *J. Cell Sci.* **92**, 37–49.
16. Brodner, A. J., Ting, R. C. & Gallo, R. C. (1981) *J. Natl. Cancer Inst.* **67**, 1025–1030.
17. Burres, N. S. & Cass, C. E. (1987) *Cancer Res.* **47**, 5059–5064.
18. Sokoloski, J. A., Beardsley, G. P. & Sartorelli, A. C. (1989) *Cancer Commun.* **1**, 199–207.
19. Weinstein, G. D., Jeffes, E. & McCullough, J. L. (1990) *J. Invest. Dermatol.* **95**, 49S–52S.
20. Schwartz, P. M. & Milstone, L. M. (1989) *J. Invest. Dermatol.* **93**, 523–527.
21. Schwartz, P. M., Barnett, S. K. & Reuveni, H. (1991) *J. Invest. Dermatol.*, in press.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Hough-Monroe, L. M. & Milstone, L. M. (1991) *Anal. Biochem.*, in press.
24. Boyce, S. T. & Ham, R. G. (1983) *J. Invest. Dermatol.* **81**, 33S–40S.
25. Albers, K. M. & Taichman, L. B. (1984) *J. Invest. Dermatol.* **82**, 161–164.
26. Pittelkow, M. R., Wille, J. J. & Scott, R. E. (1986) *J. Invest. Dermatol.* **86**, 410–417.
27. Wilke, M. S., Hsu, B. M., Wille, J. J., Pittelkow, M. R. & Scott, R. E. (1988) *Am. J. Pathol.* **131**, 171–181.
28. Watt, F. M., Jordan, P. W. & O'Neill, C. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5576–5580.
29. Ross, D. W. (1976) *Cell Tissue Kinet.* **9**, 379–387.
30. Taichman, L. B. & Prokop, C. A. (1982) *J. Invest. Dermatol.* **78**, 464–467.
31. Meuth, M. (1989) *Exp. Cell Res.* **181**, 305–316.
32. Reichard, P. (1987) *Biochemistry* **26**, 3245–3248.
33. Weinstein, G. D., Goldfaden, G. & Frost, P. (1971) *Arch. Dermatol.* **104**, 236–243.
34. Newburger, A. E., Weinstein, G. D. & McCullough, J. L. (1978) *J. Invest. Dermatol.* **70**, 183–186.
35. Newton, J. A., Camplejohn, R. S., Bowyer, C., McGibbon, D. H. & Wright, N. A. (1985) *Dermatologica* **171**, 469–473.