# INDUCTION OF TISSUE TRANSGLUTAMINASE IN HUMAN PERIPHERAL BLOOD MONOCYTES

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Transglutaminases are a group of enzymes that catalyze the covalent conjugation of amines to protein-bound glutamine residues. The products of transglutaminase activity are proteins covalently cross-linked by $\epsilon$-($\gamma$-glutaminyl)-lysine isopeptide bonds and proteins containing covalently conjugated polyamines (1–6). Several distinct intracellular and extracellular transglutaminases have been identified (1, 2). The predominant form of this enzyme in liver and cultured fibroblasts is tissue transglutaminase, an ~80,000 D polypeptide (2, 7–9). We have measured the amounts of this enzyme in a variety of normal and transformed cell types and have found that macrophages can contain extremely high levels (9).

Studies from several laboratories have indicated that transglutaminase may play an important role in macrophage function. Activation of guinea pig and mouse macrophages in vivo is associated with marked increases in transglutaminase activity due to a dramatic induction of tissue transglutaminase (9–11). In both cell types, elevated levels of transglutaminase activity have been correlated with enhanced Fc receptor-mediated phagocytic capacity (10–12). Furthermore, several inhibitors of transglutaminase activity block phagocytosis of immune complexes and opsonized erythrocytes (11, 12). However, the precise role of transglutaminase in macrophage function remains to be determined.

Peripheral blood monocytes can differentiate into macrophages when cultured in vitro (13–18). The differentiation of myelobastic cells into macrophages has been associated with marked increases in the levels of transglutaminase (10, 19). Therefore, we were interested in determining the level of tissue transglutaminase in peripheral blood monocytes and if differentiation of these cells into macrophages was associated with alterations in the level of the enzyme. We report here that the levels of tissue transglutaminase in freshly isolated human peripheral blood monocytes are very low but that the enzyme accumulates to significant levels during culture of these cells in vitro.

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## Materials and Methods

*Materials.* Dulbecco's high glucose minimal essential media (DMEM)[1] was obtained from Gibco Laboratories, Grand Island, NY and was free of endotoxin at the level of 0.16 ng/ml based on the *Limulus* amebocyte lysate assay (Associates of Cape Cod, Wood's Hole, MA). WI-38 human embryo lung fibroblasts and A431 human epidermoid carcinoma cells were obtained from the American Type Culture Collection, Bethesda, MD. Human platelets were obtained from whole blood by differential centrifugation. Lipopolysaccharide (LPS) B, *Escherichia coli* 0111:B4 was obtained from Difco Laboratories, Inc., Detroit, MI. Cycloheximide, N,N-dimethylated casein, and actinomycin D were purchased from Calbiochem-Behring Corp., San Diego, CA; $Na^{125}I$ and [$^3$H]dansylcadaverine were from Amersham Corp., Arlington Heights, IL.

*Monocyte Isolation and Culture.* Heparinized blood was obtained from healthy human volunteer donors. Mononuclear cells were isolated by density gradient centrifugation in Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) or Lymphoprep (Nyegaard, Oslo, Norway). The cells were suspended at $10 \times 10^6$ cells/ml in DMEM containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated autologous serum. 1 ml was plated onto 35-mm Falcon tissue culture dishes (Becton-Dickinson Labware, Oxnard, CA). After 60 min, nonadherent cells were removed by washing three times with DMEM. The adherent cells were >90% monocytes as determined by the presence of granules staining for peroxidase and nonspecific esterase, uptake of latex particles, and the presence of Fc and C3 receptors (20). The adherent monocytes then were cultured for appropriate times at 37°C in an atmosphere of 5% $CO_2$-air.

For suspension culture on a Teflon-coated surface, mononuclear cells were incubated in 60-mm Falcon dishes lined with Teflon FEP, 100 Å thick, held in place by a tightly fitting Teflon ring (20). $20 \times 10^6$ mononuclear cells in 8 ml DMEM with 10% heat-inactivated autologous serum were placed in each dish. After 3, 6, or 10 d, the suspended cells were harvested and the concentration was adjusted to $10 \times 10^6$ cells/ml in DMEM/10% autologous serum. 1 ml was placed in 35-mm Falcon dishes and the adherent cells were isolated as described above. In both adherent and suspension cell cultures, the medium was changed at 3-d intervals.

*Determination of Transglutaminase Activity.* Cultured monocytes were washed three times at 37°C with Tris-buffered normal saline (20 mM Tris-HCl, 0.15 M NaCl, pH 7.5) and scraped from the dish in the same buffer containing 1 mM EDTA and 15 mM β-mercaptoethanol. Cells were disrupted by sonication, and transglutaminase activity in the lysates was determined by the $Ca^{2+}$-dependent incorporation of [$^3$H]dansylcadaverine into dimethylated casein, as described previously (9, 21). The protein concentration of cell lysates was determined by a Coomassie blue binding assay using bovine gamma globulin as the standard.

*Immunochemical Detection of Tissue Transglutaminase.* To detect tissue transglutaminase in cell extracts, cells were washed, removed from the dish, and sonicated as described above. Cell lysates were solubilized in 20 mM sodium phosphate, pH 6.8, with 1% sodium dodecyl sulfate (SDS), 0.75 M β-mercaptoethanol, 2.5% sucrose, and 0.001% bromphenol blue, and were boiled for 3 min. Solubilized extracts were fractionated by electrophoresis on a 6.5% polyacrylamide gel and electroblotted onto nitrocellulose paper (Schleicher and Scheull, Inc., Keene, NH). The paper was neutralized with 5% bovine serum albumin and tissue transglutaminase was detected by autoradiography using a monospecific $^{125}$I-antitransglutaminase antibody (9). To quantify the amount of tissue transglutaminase in cell extracts, the density of the transglutaminase band on autoradiograms was determined using a laser densitometer (LKB, Uppsala, Sweden) and compared with standards derived from purified guinea pig liver tissue transglutaminase run in parallel lanes on the same gel.

*Microscopy.* Cultured monocytes were photographed using an Olympus IM inverted microscope (Olympus Camera Corp., Tokyo, Japan) with phase-contrast optics and Polaroid 084 film (ASA 3000).

[1] *Abbreviations used in this paper*: DMEM, Dulbecco's modified essential medium; LPS, bacterial lipopolysaccharide; SDS, sodium dodecyl sulfate.

## Results

*Transglutaminase in Freshly Isolated and Cultured Monocytes.* Cell extracts from freshly isolated monocytes were fractionated by SDS-gel electrophoresis and probed with an iodinated antibody to tissue transglutaminase. A single immunoreactive band was detected, possessing an electrophoretic mobility slightly slower than purified guinea pig liver transglutaminase (Fig. 1, lanes 1 and 2). The mobility of tissue transglutaminase in monocyte extracts was the same as in extracts of other human tissues and cells (Figs. 1, lanes 3–5). Quantitation of the amount of immunoreactive transglutaminase in monocytes by densitometric scanning of autoradiograms indicated that the concentration of enzyme was <20 ng/mg cell protein. Measurements of enzyme activity in 10 different preparations of freshly isolated monocytes gave a value of 0.7 ± 0.1 pmol/min/mg cell protein (mean ± SEM).

Adherent culture of human monocytes for up to 10 d was associated with a progressive and marked increase is transglutaminase activity (Fig. 2*A*). The increase in enzyme activity was not linear but accumulated more rapidly after 2–3 d of culture. In the experiments shown in Fig. 2, over a 10-d culture period, transglutaminase activity increased 46-fold, from 0.9 to 42 pmol/min/mg. This increase in enzyme activity was not due to activation of preexisting enzyme but rather to the accumulation of tissue transglutaminase in the cells. As shown in Fig. 2*B*, extracts of cells cultured for 4 or 7 d in autologous serum showed increased levels of tissue transglutaminase (82,000 D) that paralleled the increase in enzyme activity.

There was some variation among donors both in the basal levels of transglutaminase activity and in the pattern of accumulation of the enzyme in cultured cells. Fig. 3 demonstrates individual results from 10 different experiments using seven different donors. Basal values of enzyme activity ranged from 0.2 to 1.4 pmol/min/mg cell protein. After 2 d of culture in autologous serum, all of the

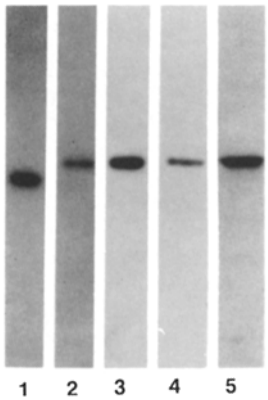


FIGURE 1. Western blots of tissue transglutaminase in human cell extracts, as described in Materials and Methods. (Lane 1) 1 ng guinea pig liver tissue transglutaminase, $M_r$ = 80,000 (48 h exposure); (lane 2) 20 μg of peripheral blood monocytes cultured 2 d in autologous serum (48 h exposure); (lane 3) 100 μg of human embryo lung fibroblasts (WI-38 cells, 18 h exposure); (lane 4) 50 μg of human platelets (24 h exposure); and (lane 5) 100 μg of human epidermoid carcinoma cells (A431 cells, 18 h exposure).

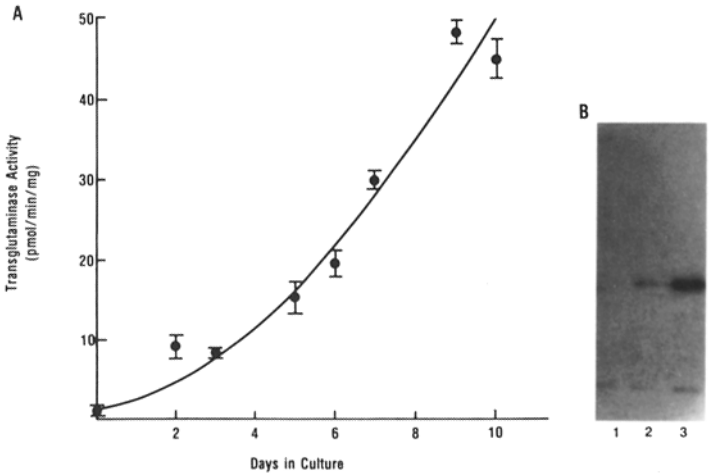


FIGURE 2. Time course of accumulation of transglutaminase in cultured monocytes. (*A*) Monocytes were cultured on plastic tissue-culture dishes as described in Materials and Methods. At the indicated times, cells were washed, sonicated, and assayed for transglutaminase activity. Values shown are the mean ± SEM of 3–6 determinations from individual dishes. (*B*) Monocytes attached to plastic tissue-culture dishes were incubated in 10% autologous serum for 1 h (lane 1), 4 d (lane 2), or 7 d (lane 3). Samples containing 10 μg of protein were subjected to Western blot analysis as described in Materials and Methods.

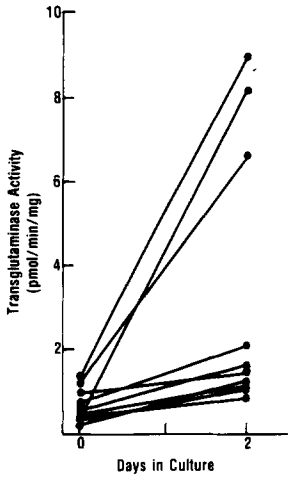


FIGURE 3. Variable accumulation of transglutaminase activity in cultured human monocytes. Monocytes isolated from different donors ($n$ = 7) and on different occasions ($n$ = 10) were assayed for transglutaminase activity immediately or after 48 h of culture in 10% autologous serum. Values shown represent the mean of three determinations on single samples taken from individual experiments.

cell preparations showed increased levels of enzyme activity but some were elevated more than others. The variability in the extent of increase in enzyme activity was even more pronounced at later times (data not shown).

*Changes in Morphology of Cultured Monocytes.* The appearance of tissue transglutaminase was paralleled by a change in the morphology of cultured monocytes. Freshly isolated cells were generally rounded in appearance whereas cells cul-

tured for 7 d were spread and flattened, with an appearance typical of macrophages. As previously shown, a 50% loss in cell number occurred during the first 24 h in culture, with the cell number remaining constant after this time (20). In the experiment shown in Fig. 2*A*, the total protein increased from 145 μg per dish at day 2 to 220 μg per dish at day 10. Therefore, the marked increase in transglutaminase specific activity observed over this time period does not reflect preferential selection of a subset of monocytes with high levels of enzyme activity.

*Culture of Monocytes on a Teflon Surface.* The degree of increase in tissue transglutaminase was dependent on culture conditions. Cells cultured in Teflon-coated dishes, where adhesion of cells to substrate is minimized, showed much less increase in transglutaminase than did cells cultured in plastic tissue-culture dishes (Table I). The level of tissue transglutaminase increased from <10 ng/mg cell protein on day 0 to 1,010 ng/mg cell protein on day 10 in cells cultured on plastic dishes whereas it increased to only 375 ng/mg cell protein in cells cultured on Teflon. Similarly, the enzyme activity increased ~50-fold in the adherent cells but only 20-fold in the cells cultured on Teflon (Table I). The level of tissue transglutaminase in 10-d-old cultured cells was 0.1% of total cellular protein.

*Acceleration of Transglutaminse Increase by LPS.* LPS induces a variety of differentiated functions in monocytes and macrophages (22). Culture of paired samples of monocytes from an individual donor in media with LPS (1 μg/ml) resulted in a fivefold enhancement in the level of transglutaminase activity (Fig. 4). The more rapid accumulation of tissue transglutaminase was observed after both 2 and 4 d of culture. Prolonged culture of human monocytes in LPS-containing media appeared to decrease cell viability (data not shown) and so the effect of prolonged culture in LPS on enzyme accumulation could not be measured.

The concentration range over which LPS was effective is shown in Fig. 5. In

TABLE I

*Effect of Adherence on the Induction of Transglutaminase in Cultured Monocytes**

| Days in culture | Transglutaminase activity‡ | | Tissue transglutaminase levels§ | |
|---|---|---|---|---|
| | Plastic | Teflon | Plastic | Teflon |
| 0 | 1.2 ± 0.3 | — | ND | — |
| 3 | 8.3 ± 0.3 | 7.1 ± 0.1 | 100 | 25 |
| 6 | 19.6 ± 1.6 | 10.1 ± 4.1 | 550 | 100 |
| 10 | 44.8 ± 2.4 | 22.4 ± 1.5 | 1,010 | 375 |

* Adherent monocytes were cultured in DMEM with 10% autologous serum in plastic tissue-culture dishes with or without a Teflon lining for 3, 6, or 10 d. Media was changed at 3-d intervals. Aliquots of mononuclear leukocytes were cultured in suspension in Teflon-lined tissue culture dishes as described in Materials and Methods. Media was changed at 3-d intervals. At the indicated intervals, the cells were harvested and mononuclear phagocytes isolated by adhesion to plastic tissue-culture dishes without Teflon for 1 h.

‡ Activity is expressed as pmol/min/mg. Values shown represent the mean ± SEM of six determinations.

§ Transglutaminase levels are expressed as ng/mg cell protein. Data were determined by quantitative Western blotting as described in Materials and Methods. ND, not detectable.

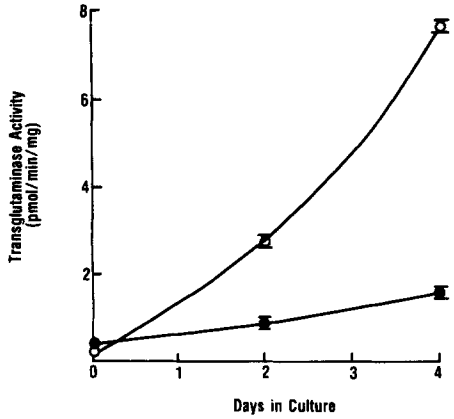


FIGURE 4. Effect of LPS on the expression of transglutaminase activity in cultured monocytes. Cells were cultured in 10% autologous serum in the presence (○) or absence (●) of 1 μg/ml bacterial LPS for varying lengths of time. Values shown represent the mean and SEM of six determinations from individual dishes.

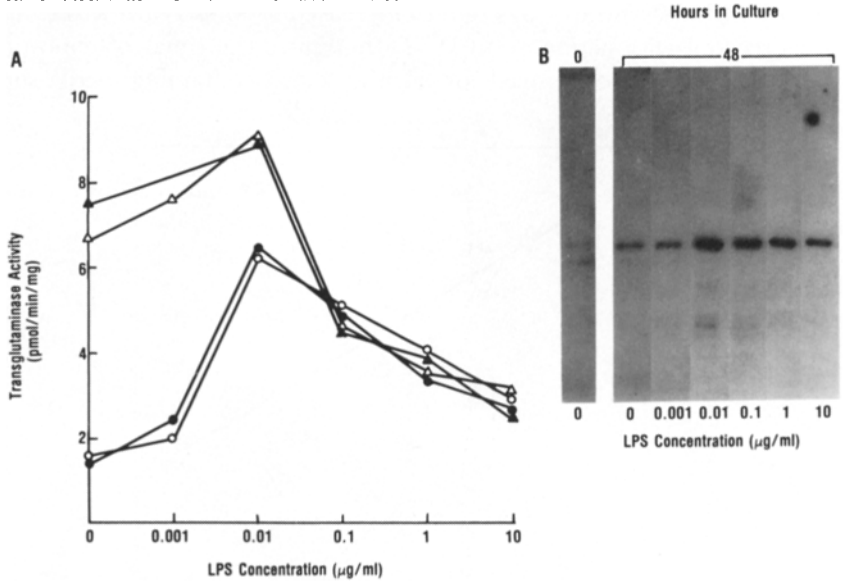


FIGURE 5. Effect of LPS concentration on the levels of transglutaminase in cultured monocytes. (*A*) Monocytes were collected from four donors and cultured in different concentrations of LPS. After 48 h, cells were harvested for determination of transglutaminase activity. Values shown represent the mean of six determinations from individual donors. (●, ○, ▲, △) Results from four independent experiments using cells obtained from different donors. (*B*) Western blot analysis of the levels of tissue transglutaminase in monocytes after 48-h culture in various concentrations of LPS. Each lane contains 20 μg of cell extract from samples shown in *A* by the open circles.

all donor cells examined, 10 ng/ml appeared to be an optimal concentration for increasing the levels of transglutaminase activity; higher concentrations of LPS were less effective. As previously noted, the response to LPS appeared to vary among donors. Monocytes that rapidly accumulated transglutaminase in the absence of LPS showed little further response when LPS was included in the culture media (Fig. 5*A*, triangles). Cells that showed less of a response to culture

in serum alone were much more sensitive to LPS (Fig. 5*A*, circles). Western blots of LPS-treated cells with the antitransglutaminase antibody demonstrated that the effect of LPS on transglutaminase activity in monocytes was due to an increased level of the enzyme and not to an activation of preexisting enzyme (Fig. 5*B*).

*Induction of Transglutaminase Synthesis in Cultured Monocytes.* To define the molecular mechanism underlying the accumulation of tissue transglutaminase in cultured monocytes, the half-life of the enzyme in cells cultured for 2 or 10 d in autologous serum was examined. After treatment with cycloheximide, the enzyme in 2-d-old cells possessing low levels of transglutaminase had a half-life of 11 h (Fig. 6). The half-life of the enzyme was approximately the same (7 h) in monocytes cultured for 10 d.

The finding of increased levels of transglutaminase in cultured monocytes in the absence of a decrease in the rate of enzyme degradation indicated that the rate of transglutaminase synthesis had been increased. To determine if the induction of enzyme synthesis was due to increased synthesis of mRNA, monocytes were treated with actinomycin D. To minimize the times of exposure to actinomycin D, cells were cultured for 24 h in serum-containing media supple-

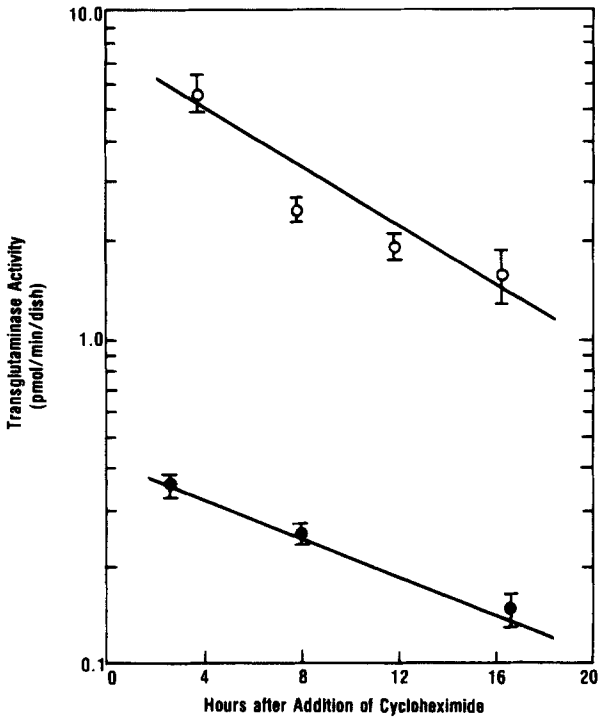


FIGURE 6. Transglutaminase activity in cultured monocytes treated with cycloheximide. Monocytes in plastic tissue-culture dishes were incubated for 2 (○) or 10 (●) d in 10% autologous serum before 50 μM cycloheximide was added to the media. At the indicated times after addition of cycloheximide, the cells were removed from the dish and the amount of transglutaminase activity was determined. Values shown represent the mean ± SEM based upon six determinations.

TABLE II
*Effect of Actinomycin D on the Induction of Transglutaminase in Cultured Monocytes*

| Monocytes* | Transglutaminase activity |
|---|---|
| | *pmol/min/mg* |
| Freshly isolated cells | 0.5 ± 0.1 |
| 2-d-old monocytes without actinomycin D | 8.2 ± 0.3 |
| 2-d-old monocytes exposed to actinomycin D for 24 h | 1.0 ± 0.2 |

* Monocytes were cultured 24 h in 10% autologous serum containing 10 ng/ml LPS. Cells were then washed and the culture was continued for an additional 24 h in the same media (control) or the same media plus actinomycin D (0.5 μg/ml). Data shown are the mean ± SEM from six determinations.

mented with 10 ng/ml LPS. After this preincubation, the cells were cultured for an additional 24 h in the presence or absence of actinomycin D (0.5 μg/ml). This level of the drug was sufficient to inhibit [$^3$H]uridine incorporation into RNA by 95% (data not shown). As shown in Table II, the transglutaminase activity in control cells increased from 0.5 to 8.2 pmol/min/mg cell protein, whereas in actinomycin D-treated cells the enzyme activity was only 1.0 pmol/min/mg cell protein. Western blot analysis showed a corresponding lack of the enzyme in actinomycin D-treated cells (data not shown).

## Discussion

The results of the studies reported in this paper demonstrate that the level of tissue transglutaminase in freshly isolated human monocytes is low, <20 ng per mg cell protein. The enzyme increased 50-fold during 10-d culture of the cells in autologous serum, reaching 0.1% of total cell protein. LPS accelerated the accumulation of the enzyme in cultured monocytes. The elevated level of tissue transglutaminase in these cells appears to be due to increased gene transcription and induction of enzyme synthesis, rather than to enzyme activation or to a decreased rate of enzyme degradation.

We have recently reported (9) that culture of resident murine peritoneal macrophages in serum-containing media produces a large increase in the levels of tissue transglutaminase due to increased transglutaminase gene expression. The induction of the enzyme in human monocytes also appears to involve an elevated rate of transglutaminase gene transcription. However, the pattern of enzyme induction in human monocytes is quite different from that in murine macrophages. In murine macrophages there is a very rapid induction of the enzyme. The level of transglutaminase is increased within 2 h of exposure of the cells to serum and can increase 150-fold within 24 h. In cultured monocytes, the induction of enzyme synthesis is more gradual, developing over several days, and the accumulation of the enzyme is less dramatic than in the macrophages. We believe that the induction of transglutaminase in monocytes reflects the differentiation of these cells into macrophages whereas the induction of the enzyme in macrophages is due to a specific activation of transglutaminase gene expres-

sion.

The induction of tissue transglutaminase in human monocytes appears to be linked to in vitro culture of the cells. Culture of peripheral blood monocytes in serum-containing media is thought to induce differentiation into macrophages. In vitro-derived human macrophages exhibit altered levels of several enzymes, including increases in plasma membrane-bound 5′-nucleotidase (13), lysozyme secretion (14), acid phosphatase (14), and plasminogen activator secretion (16), and a decrease in lysosomal peroxidase (13). The production of oxygen radicals is decreased but can be restored by stimulation with lymphokines or bacterial endotoxin (23, 24). Peritoneal macrophages, alveolar macrophages, and Kupffer cells from various animal species have relatively high levels of tissue transglutaminase, 200–1,000 ng/mg cell protein (9, and unpublished observations). Therefore, the increased levels of enzyme in the cultured human monocytes may reflect their maturation into macrophages. The differentiation of murine myeloblastic leukemia cells into macrophages is also associated with a marked accumulation of transglutaminase (19). Thus, the induction of tissue transglutaminase may be a feature of the terminal differentiation of myelocytic cells into macrophages.

We have found two experimental manipulations that modulated the rate of transglutaminase induction in cultured monocytes. LPS accelerated the induction of the enzyme and suspension culture retarded it. LPS has been reported to promote the expression of differentiated functions in macrophages and monocytes (22, 25–27) and to induce transglutaminase in differentiating myeloblastic cells (19). On the other hand, suspension culture has been reported to delay the differentiation of monocytes into macrophages (18). Therefore, the observed effects of these manipulations on transglutaminase induction may be secondary to the effects on differentiation.

The function of transglutaminase in monocytes and macrophages is not well understood. It has been suggested that the enzyme might play a role in Fc receptor-mediated phagocytosis in macrophages (10–12, 28, 29). The degree of phagocytosis of IgG-coated erythrocytes correlates directly with the level of enzyme activity in murine macrophages (10), and inhibitors of transglutaminase block Fc receptor-mediated phagocytosis in guinea pig peritoneal macrophages (11). Phagocytosis of IgG-coated erythrocytes activates transglutaminase in rat peritoneal macrophages (12). Furthermore, transglutaminase can aggregate purified Fc receptors from human mononuclear leukocytes, which suggests a role for the enzyme in the regulation of Fc receptor function (28). However, the fact that freshly isolated monocytes possessing low levels of transglutaminase are fuly capable of Fc receptor-mediated phagocytosis (17, 30) indicates that a high level of the enzyme is not essential for this activity. Because the capacity of monocytes to phagocytose IgG-coated erythrocytes is increased by culture (30), the role of transglutaminase in Fc receptor-mediated phagocytosis in monocytes may be facilitative rather than essential. In contrast to Fc receptor-mediated phagocytosis, interiorization mediated by the complement receptor is expressed only after prolonged culture of human monocytes (17, 30, 31). The induction of this activity parallels the induction of tissue transglutaminase, suggesting that the enzyme might play a necessary role in complement receptor-mediated phagocytosis in monocytes.

## Summary

The levels and activity of tissue transglutaminase were studied in human peripheral blood monocytes during differentiation into macrophages in vitro. The enzyme was present at low levels in freshly isolated monocytes (<20 ng/mg cell protein) but increased 50-fold during 10 d of adherent culture in autologous serum, reaching levels of 0.1% of total cellular protein. The rate of appearance of tissue transglutaminase in monocytes was accelerated by low levels of lipopolysaccharide. The half-life of disappearance of transglutaminase from human monocytes was 11 and 7 h in 2-d-old and 10-d-old cells, respectively. Treatment of 1-day-old monocytes with actinomycin D for 24 h blocked the increase in transglutaminase levels. These results indicated that the induction of gene transcription and protein synthesis was responsible for the increased transglutaminase levels and activity observed with cultured human monocytes. The induction of tissue transglutaminase may be a component in the in vivo differentiation of human monocytes into macrophages.

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