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# INDUCTION OF ANGIOTENSIN CONVERTING ENZYME IN HUMAN MONOCYTES IN CULTURE

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SUMMARY: Angiotensin converting enzyme (E.C.3.4.15.1, peptidyl dipeptidase) in circulating human monocytes rose from undetectable or minimal levels in vivo to as high as 35.5 nmol/min·mg protein (>300-fold increase) after 6 or 7 days in culture. Enzyme induction was enhanced by autologous serum and exposure for two days to 0.45 μM dexamethasone. Potent inhibition of enzyme induction by 370 μg/ml of actinomycin D and 1 μM cycloheximide suggested that new messenger RNA and enzyme biosynthesis are involved in the induction. Human monocyte and lung enzyme were similar with respect to EDTA inhibition, $CoCl_2$ activation and inhibition by an anti-enzyme antiserum. Human lymphocytes had minimal or undetectable enzyme which was not induced after 4 days in culture.

The enzymatic and other biochemical functions of mononuclear phagocytes, which have long been investigated for their phagocytic and immunologic role, have recently received increasing recognition (1). The recent association of strikingly increased angiotensin converting enzyme (E.C.3.4.15.1, peptidyl dipeptidase) (2,3) in serum (4-6) and granulomatous lymph nodes (6) in sarcoidosis, in serum and spleen in Gaucher's disease (7-9) and in serum in leprosy (10) suggests the possibility that epithelioid cells in sarcoidosis, the macrophages in leprosy and Gaucher cells may be actively synthesizing ACE (5,11). Recent immunofluorescent localization of angiotensin II, the biologically potent (pressor) ACE-catalyzed cleavage product of the decapeptide angiotensin I (12), and ACE (13) in epithelioid cells of sarcoidosis granulomas has supported this possibility. However, ACE is generally barely detectable in mononuclear phagocytes and experimental Freund's adjuvant granulomas (6,8,14) suggesting that specific control mechanisms may be involved in its biosynthesis (11).

Abbreviations: ACE, angiotensin converting enzyme; PBSD, Dulbecco's calcium and magnesium deficient phosphate buffered saline.

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Glucocorticosteroids specifically were found to induce the biosynthesis of ACE in rabbit alveolar macrophages in culture (15,16).

Since monocytes are believed to be precursors of macrophages, and epithelioid and Gaucher cells (1), their potential for ACE synthesis was investigated. The results indicate that, while circulating human monocytes and lymphocytes *in vivo* possess only barely detectable ACE activity, the monocytes, but not the lymphocytes, under the culture conditions employed, have the capacity for a greater than 300-fold induction in ACE biosynthesis which is dependent on *de novo* synthesis of RNA.

## MATERIALS AND METHODS

Seven ml portions of human blood from normal individuals which had been diluted 1:1 on collection with PBSD containing 10 units/ml of heparin were layered over 3 ml of Ficoll-Hypaque (Pharmacia) and centrifuged at 400 g for 30 min at 15-20°. The interphase mononuclear fraction was diluted with an equal volume of PBSD, collected by centrifugation for 20 min at 570 g and washed 3 times in PBSD with centrifugation. A monocyte monolayer was obtained by the method of Hovi, et al (17) and cultured at $1x10^7$ cells/plate in 1 ml of a 1:1 mixture of medium 199 and RPMI 1640 containing, in some cases, 20% autologous serum which had been heated at 60° for 30 min. The cells in culture generally became flattened, enlarged and put out processes.

On the sixth day of incubation after two days of exposure of some cultures to 0.45 μM dexamethasone (15) the cells were harvested with a rubber "policeman" and collected by centrifugation at 570 g for 10 min. In some cases, the few nonadherent cells in the medium were also collected. All cells were washed with 3 ml of PBSD and suspended in 0.1 ml of 50 mM potassium phosphate, pH 8.3. Cells and media were kept at -83° until assay as previously described (15,18). Protein was assayed by the method of Lowry, et al (19).

## RESULTS

The specific activity of ACE in monocytes in an adherent monolayer after 6 days in culture rose 60-fold in the absence of serum and 69-fold in the presence of serum in a representative experiment (Table I). The presence of 0.45 μM dexamethasone stimulated a further two to three-fold increase in ACE specific activity to a 146-fold increase from initial levels in the absence of serum and a 202-fold increase in the presence of serum (Table I). The total activity recovered from cells and culture medium similarly increased strikingly (35-fold in the absence of serum, 162-fold in the presence of serum, 80-fold in the presence of dexamethasone and 254-

TABLE I

Angiotensin Converting Enzyme Activity in Monocytes in Culture*

| | No Serum | | 20% Serum | |
|---|---|---|---|---|
| | Control | Dexamethasone | Control | Dexamethasone |
| | Cells, nmols·min$^{-1}$·mg protein$^{-1}$ | | | |
| Initial | 0.12 | 0.12 | 0.12 | 0.12 |
| 6 days attached | 7.25 | 17.6±0.1‡ | 8.3±1.0 | 24.3±6.2 |
| 6 days unattached | 0.03 | 6.4±2.4 | 0.76±0.31 | 3.9±0.5 |
| | Total (cells + medium), nmol·min$^{-1}$·culture$^{-1}$ | | | |
| Initial | 0.02 | 0.02 | 0.02 | 0.02 |
| 6 days | 0.70 | 1.61±.10 | 3.24±.37 | 5.08±.86 |

* 1x10$^7$ cells/plate
‡ Standard error of the mean

fold in the presence of both dexamethasone and serum) (Table I). The highest specific activity observed was 35.5 nmol/min·mg protein (>300-fold increase) after 7 days in culture.

The small number of cells which became non-adherent and recoverable in the monocyte culture medium had no or very little increase in ACE specific activity in the absence of dexamethasone, but were markedly stimulated by dexamethasone to a 30 to 50-fold increase in ACE specific activity (Table I). These cells were predominantly small and resembled lymphocytes, but a minority of large cells of the monocyte-macrophage type were also present and may have been responsible for the significantly increased ACE. Lymphocytes after almost 4 days in culture had only low levels of activity which was not increased in the presence of dexamethasone (Table II).

The increase in ACE between 3 and 5 days in culture in the presence

TABLE II

Angiotensin Converting Enzyme Activity

in Lymphocytes in Culture

| Treatment | Time in culture, hrs | Enzyme Activity $nmol \cdot min^{-1} \cdot mg\ protein^{-1} \pm SEM$ |
|---|---|---|
| None | 0 | 0 |
| None | 89.5 | 0.50±.09, n=3 |
| 0.45 μM dexamethasone | 89.5 | 0.38±.12, n=3 |

$4x10^6$ non-adherent lymphocytes were cultured in test tubes in 2 ml of RPMI 1640 containing 15% autologous serum at 37° in 5% $CO_2$. Dexamethasone or ethanol vehicle alone (15) were added for the last 66.5 hrs of culture.

of 0.45 μM dexamethasone and serum was inhibited 92.4% by 1 μM cycloheximide, indicating that new enzyme synthesis was involved in the increased ACE activity. A 3.5 hr pulse with 370 ng/ml of actinomycin D and 0.47 μM dexamethasone after 3 days in culture in the presence of serum resulted in a 69% inhibition of the increase in ACE activity from 3 to 5 days in monocytes cultured during this period with the addition of 0.45 μM dexamethasone, suggesting that new messenger RNA synthesis is involved in the increased ACE activity.

The ACE synthesized by the monocytes was similar to human lung and serum ACE with respect to inhibition by EDTA and activation by $CoCl_2$ (Table III) (6,20). The potent inhibition of monocyte ACE catalytic activity by a rabbit anti-human lung ACE which we have prepared with the aid of purified human lung ACE (unpublished observations) (81.6%) was similar to that obtained with human lung ACE (82.3%).

## DISCUSSION

The present work establishes ACE as another enzyme which is under stringent control in human circulating monocytes. The results indicate

TABLE III

Inhibition and Activation of Monocyte

Angiotensin Converting Enzyme

| | Enzyme Activity, $nmol \cdot min^{-1} \cdot ml^{-1}$ | | |
|---|---|---|---|
| ACE | Control | EDTA, 1mM | EDTA, 0.5mM $CoCl_2$, 1mM |
| Medium* | 18.5 | 0.80(4)‡ | 27.2 (147) |
| Monocytes | 14.6 | 0.79 (5) | 23.9 (164) |

* Obtained from the cell cultures and concentrated 5-fold by ultrafiltration through a millipore PTGC filter
‡ % of control

that while human circulating monocytes *in vivo* contain only barely detectable ACE activity they nevertheless possess the capacity for induction of intense, RNA synthesis-dependent ACE synthesis to specific activities higher than those present in human lung (6). The function of this potentiality for massive increase in ACE is not known, but it is possible that it may serve as a signal for other cells, such as lymphocytes, and play a role in cellular defense against biological or chemical agents. It is not known whether ACE may be normally or pathologically induced in human monocytes *in vivo*, nor what the inducer(s) may be.

The demonstrated capability of cultured monocytes for vastly increased ACE synthesis and the presence in the pathologic lesions of sarcoidosis, Gaucher's disease and leprosy of epithelioid and Gaucher cells, and macrophages, which are believed to be derived from monocytes (1), suggests that these cells are somehow induced to synthesize greatly increased quantities of ACE, resulting in the increased ACE present in the circulation, granulomas and spleen in sarcoidosis (4-6) and Gaucher's disease (7-9) and circulation in leprosy (10). It is not clear whether there is any relationship between the pathological association of ACE with

these diseases and its possible normal physiological function in monocytes. The possible involvement of ACE in the development of these diseases is not known, but a role in tissue catabolism and fibrotic replacement is conceivable. The inducing event in these conditions is the subject of further investigation. The relationship between the apparent turning on of ACE synthesis in sarcoidosis lesions and the etiology of the disease is not clear, but ACE appears to be a useful probe of the pathogenesis of the disease (5,6,11).

Study is underway to clarify whether the enhancement of ACE synthesis by autologous serum is due to its glucocorticosteroid content or to other factors and whether the ability of human monocytes to increase ACE significantly in the absence of added glucocorticosteroid (in contrast to rabbit alveolar macrophages) may be due to the strong adherence of the monocytes, residual inducer activity, or other factors.

Lysozyme has generally been found to be synthesized constitutively, while the activity of collagenase, elastase and plasminogen activator increases, and 5'-nucleotidase decreases with increasing physiological activity of mononuclear phagocytes (1). In monolayer culture human monocyte myeloperoxidase activity greatly diminished after 4 days, while plasma membrane associated 5'-nucleotidase increased 11-fold during the first 2 days (21). In contrast to results with 5'-nucleotidase, human monocyte ACE tended to increase after 2 days in culture; the extent of increase was more than an order of magnitude greater and the increase did not require the presence of serum, although the level of increase was influenced by it.

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## REFERENCES

1. Unanue, E.R. (1976) Am. J. Path. 83, 396-417.
2. Bakhle, Y.S. (1974) Handbook Exper. Pharmacol. 37, 41-80.
3. Soffer, R.L. (1976) Annu. Rev. Biochem. 45, 73-94.
4. Lieberman, J. (1975) Am. J. Med. 59, 365-372.

5. Silverstein, E., Friedland, J., Lyons, H.A., and Gourin, A. (1976) Ann. N.Y. Acad. Sci. 278, 498-513.
6. Silverstein, E., Friedland, J., Lyons, H.A., and Gourin, A. (1976) Proc. Natn. Acad. Sci. U.S.A. 73, 2137-2141.
7. Lieberman, J., and Beutler, E. (1976) N. Engl. J. Med. 294, 1442-1444.
8. Silverstein, E., and Friedland, J. (1977) Clin. Chim. Acta 74, 21-25.
9. Silverstein, E. (1978) Am. J. Clin. Path. 69, 467-470.
10. Lieberman, J. (1977) Ann. Int. Med. 87, 422-425.
11. Silverstein, E. (1976) Med. Hypoth. 2, 75-78.
12. Silverstein, E., Pertschuk, L.P., Friedland, J., Kim, D.S., Rosen, Y., and Onur, N. (1977) Clin. Res. 25, 668A.
13. Silverstein, E., Friedland, J., Kim, D.S., and Pertschuk, L.P. (1978) Abstract and Proceedings of the 8th International Conference on Sarcoidosis and other Granulomatous Diseases, Cardiff, in press.
14. Silverstein, E., Friedland, J., and Setton, C. (1978) Isr. J. Med. Sci. 14, 314-318.
15. Friedland, J., Setton, C., and Silverstein, E. (1977) Science 197, 64-65.
16. Friedland, J., Setton, C., Feldman, N., and Silverstein, E. (1977) Clin. Res. 25, 677A.
17. Hovi, T., Mosher, D., and Vaheri, A. (1977) J. Exper. Med. 145, 1580-1589.
18. Friedland, J., and Silverstein, E. (1976) Am. J. Clin. Path. 66, 416-424.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
20. Friedland, J., and Silverstein, E. (1976) Biochem. Med. 15, 178-185.
21. Johnson, W.D., Mei, B., and Cohn, Z.A. (1977) J. Exper. Med. 146, 1613-1626.