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The synthesis of α_2-macroglobulin by rat mammary myoepithelial cells is regulated by synergism between glucocorticoids and cytokines

Michael J. Warburton[a,*], Barry A. Gusterson[b], Michael J. O'Hare[b]

[a]*Department of Histopathology, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 ORE, UK*
[b]*The Haddow Laboratories, Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK*

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Addition of hydrocortisone to the rat mammary gland myoepithelial cell line, G4.2.3, induces the synthesis and secretion of α_2-macroglobulin. Interleukin-1β, interleukin-6 and transforming growth factor-β synergise with hydrocortisone, increasing the synthesis of α_2-macroglobulin 2- to 4-fold, although they have no effect in the absence of hydrocortisone. Interleukin-6 is the most potent inducer having an optimum concentration of 1 ng/ml. Interleukin-6, unlike interleukin-1β or tranforming growth factor-β, decreases the lag phase from 10 h to 4 h before α_2-macroglobulin synthesis is induced by hydrocortisone. These results suggest that the mechanism of activation of transcription of the α_2-macroglobulin gene in mammary myoepithelial cells might differ from that operating in hepatic cells.

Mammary gland; Myoepithelial cell; α_2-Macroglobulin; Interleukin-6

1. INTRODUCTION

In the rat, α_2-macroglobulin is an acute-phase protein. Plasma levels of α_2-macroglobulin, which is an inhibitor of all classes of proteases, rise about 100-fold following an appropriate inflammatory stimulus [1]. α_2-Macroglobulin is primarily synthesised in the liver, although extra-hepatic sources, e.g. macrophages and some fibroblast and neuronal cell lines, have been described [2–4]. Recently, we have described the induction of the synthesis of α_2-macroglobulin and an unidentified protein (p146) in an immortalised rat mammary myoepithelial cell line by hydrocortisone, a lactogenic hormone [5]. Increased production of α_2-macroglobulin during pregnancy may protect the mammary gland basement membrane and other extracellular matrix structures from excessive proteolysis.

The synthesis of acute-phase proteins is regulated by glucocorticoids and inflammatory cytokines, e.g. interleukin-1 (IL-1), IL-6 and tumour necrosis factor-α (TNFα) [6,7]. α_2-Macroglobulin synthesis is primarily induced by IL-6 and is considerably further enhanced by glucocorticoids in vivo and in vitro [8,9]. The 5′ flanking region of the rat α_2-macroglobulin gene contains both IL-6 and glucocorticoid response elements [10,11]. The combination of factors required for maximum stimulation of gene expression varies depending on the acute-phase protein and the cell line. We now report that the synthesis of α_2-macroglobulin by the rat mammary myoepithelial cell line, G4.2.3, is regulated by synergy between glucocorticoids and cytokines.

*Corresponding author. Fax: (44) (81) 767 8326.

2. MATERIALS AND METHODS

2.1. *Materials*

IL-1β and TNFα were purchased from Bachem (Saffron Walden, UK). IL-6 and TGFβ were obtained through British Biotechnology (Oxford, UK). [^{35}S]Methionine was obtained from DuPont (Stevenage, UK).

2.2. *Cell culture*

The derivation and characterisation of the rat mammary myoepithelial cell line, G4.2.3, have been described previously [5,12]. This cell line has been infected with the SV40-derived tsA58-U19 recombinant construct in the pZipneoSV(X) 1 vector [13] and shows decreased levels of nuclear T antigen, a decreased growth rate and an increase in the expression of differentiated characteristics at the non-permissive temperature (39.5°C) compared to the permissive temperature (33°C). Cells were routinely grown at 36°C and were switched to 39.5°C for 72 h before the start of experiments.

2.3. *Metabolic labelling*

Cells were metabolically labelled by growth in methionine-free medium containing 20 μCi/ml [^{35}S]methionine. The culture medium was centrifuged, proteinase inhibitors (1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM phenylmethylsulphonyl fluoride and 5 mM EDTA) added, dialysed against water and freeze-dried. ^{35}S-Labelled, secreted proteins were analysed by SDS-PAGE followed by fluorography. Autoradiograms were scanned into a Macintosh LC computer and protein bands quantified using the programme ScanAnalysis (Biosoft, Cambridge, UK).

3. RESULTS

Addition of hydrocortisone to G4.2.3 cells results in the induction of the synthesis and secretion of α_2-macroglobulin and p146 (Fig. 1). The production of these two proteins could be further increased by the addition of interleukin-6 (IL-6). IL-6 alone had no effect on the production of α_2-macroglobulin or p146 (Fig. 1). In

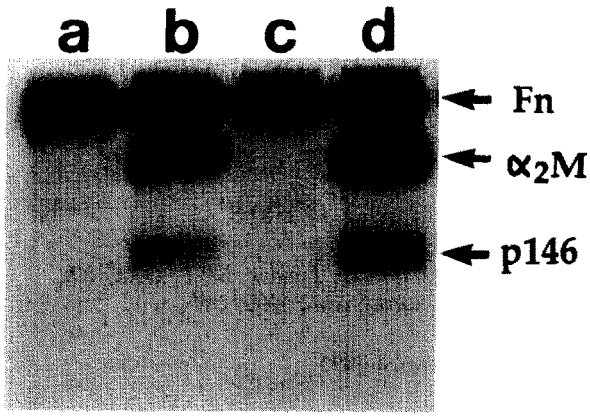

Fig. 1. SDS-PAGE of proteins secreted by G4.2.3 cells. Cells were cultured in the absence of any additions (a) or in the presence of 1 μM hydrocortisone (b), 1 ng/ml IL-6 (c) or 1 μM hydrocortisone and 1 ng/ml IL-6 (d). The medium was supplemented with [^{35}S]methionine (20 μCi/ml). After 24 h incubation, the culture medium was dialysed against water, freeze-dried and analysed by SDS-PAGE followed by fluorography. Fn, fibronectin; α_2M, α_2-macroglobulin.

addition to IL-6, other cytokines and growth factors augmented the effects of hydrocortisone (Table I). IL-1β and TGFβ both stimulated the production of α_2-macroglobulin and p146 in the presence, but not in the absence, of hydrocortisone. There was no additional synergy between these stimulatory factors. TNFα was unable to stimulate the production of the two proteins, as were the two growth factors EGF (epidermal growth factor) and bFGF (basic fibroblast growth factor) (not shown).

Dose–response curves for IL-1β, IL-6 and TGFβ demonstrated that IL-6 was the most potent factor producing a maximum response at a concentration of

Table I

Effects of cytokines on the synthesis of α_2-macroglobulin and p146 induced by hydrocortisone

Additions	Relative synthesis	
	α_2-Macroglobulin	p146
–	1.0 ± 0.1	1.0 ± 0.2
IL-1β (1 ng/ml)	2.3 ± 0.3	3.1 ± 0.3
IL-6 (1 ng/ml)	3.5 ± 0.2	5.7 ± 0.5
TNFα (1 ng/ml)	1.1 ± 0.2	1.0 ± 0.1
TGFβ (5 ng/ml)	2.2 ± 0.3	2.7 ± 0.3
IL-1β + IL-6	3.7 ± 0.2	5.7 ± 0.5
IL-1β + TGFβ	2.2 ± 0.1	3.2 ± 0.3
IL-6 + TGFβ	3.4 ± 0.3	5.5 ± 0.4
IL-1β + IL-6 + TGFβ	3.8 ± 0.2	5.6 ± 0.5

Cells were cultured in the presence of cytokines, 1 μM hydrocortisone and [^{35}S]methionine (20 μCi/ml) for 24 h. Culture medium was analysed for the secretion of α_2-macroglobulin and p146. Results are expressed as ±S.D. of 3 experiments.

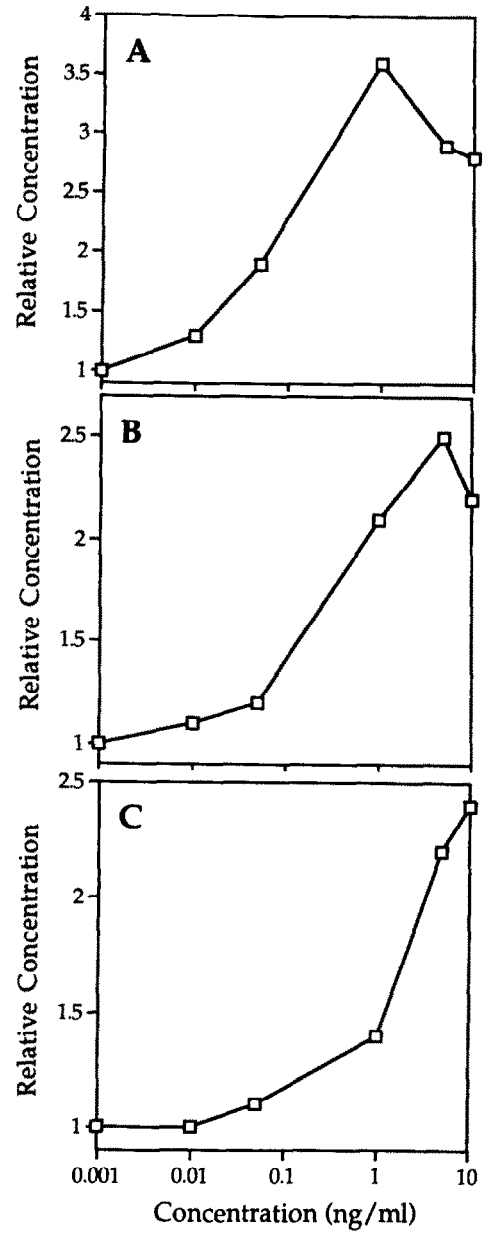

Fig. 2. Effect of cytokine concentration on the production of α_2-macroglobulin. G4.2.3 cells were cultured in the presence of 1 μM hydrocortisone and various concentrations of (A) IL-6, (B) IL-1β or (C) TGFβ and the culture medium analysed for the secretion of α_2-macroglobulin. The results are expressed as the concentration of α_2-macroglobulin relative to the concentration produced in the presence of hydrocortisone alone.

1 ng/ml (Fig. 2). IL-1β required a concentration of 5 ng/ml and TGFβ a concentration in excess of 10 ng/ml for maximum stimulation of α_2-macroglobulin production. Similar results were obtained for p146 (data not shown).

Addition of hydrocortisone to G4.2.3 cells results in the induction of α_2-macroglobulin production after a lag phase of 10–12 h (Fig. 3). Neither IL-1β nor TGFβ altered the length of the lag phase. However, IL-6 reduced the lag phase to 4–6 h. The time-course of the

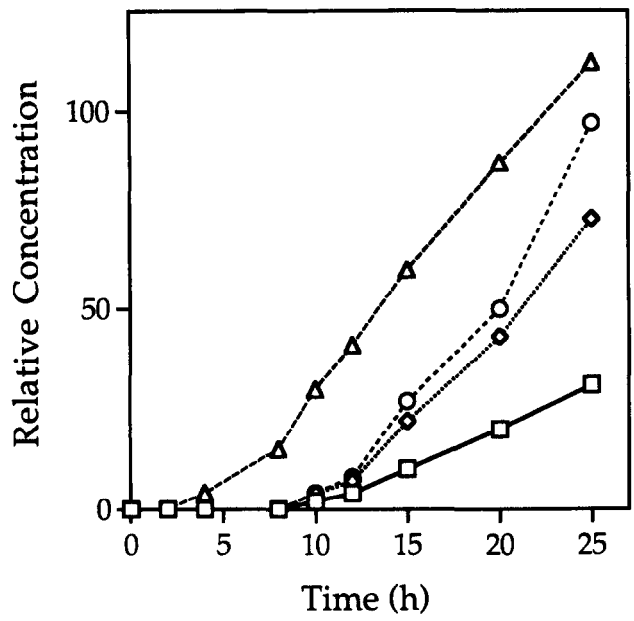

Fig. 3. Time-course of the induction of α_2-macroglobulin synthesis by hydrocortisone and cytokines. G4.2.3 cells were cultured in the presence of 1 μM hydrocortisone (□) or 1 μM hydrocortisone and 5 ng/ml IL-1β (○), 5 ng/ml TGFβ (◇) or 1 ng/ml IL-6 (△). At appropriate times, the culture medium was analysed for the presence of α_2-macroglobulin. The concentration of α_2-macroglobulin is expressed in arbitrary units.

induction of p146 production was similar to that of α_2-macroglobulin (data not shown).

4. DISCUSSION

The main function of α_2-macroglobulin is to act as an inhibitor of proteases and to clear proteases from the circulation [11]. The large size (720 kDa) of circulating α_2-macroglobulin may preclude its diffusion through extracellular matrices and may necessitate local production. During pregnancy, there is an enormous increase in the deposition of basement membrane by myoepithelial cells within the mammary gland [14]. Increased levels of hydrocortisone during pregnancy might induce the localised synthesis and secretion of α_2-macroglobulin by myoepithelial cells reducing proteolysis of the basement membranes. α_2-Macroglobulin also binds and can modify the activity of cytokines and growth factors [15]. Some of these, e.g. bFGF and TGFβ, influence patterns of growth and differentiation in the mammary gland [16].

In liver and in some hepatoma cell lines, the synthesis of α_2-macroglobulin is mainly regulated by IL-6, although glucocorticoids are additionally required for maximum stimulation [8,9]. The precise requirements vary with the cell line. For example, in H-35 hepatoma cells, neither IL-6 nor dexamethasone alone are effective but they act synergistically [17]. In FAO cells, dexamethasone alone is ineffective but greatly stimulates the induction of α_2-macroglobulin synthesis by IL-6 [10]. IL-6 alone stimulates the synthesis of α_2-macroglobulin by SH-SY 5Y neuroblastoma cells [4]. The myoepithelial cell line, G4.2.3, is somewhat unusual in that it is totally unresponsive to IL-6 alone, although IL-6 is able to synergise with glucocorticoids. These results suggest quantitative and/or qualitative differences in the levels of transcription factors that regulate the response to IL-6. A possible explanation for the effectiveness of glucocorticoids in the absence of IL-6 is that levels of IL-6 inducible transcription factors, e.g. IL6 RE-BPs [9,10], are already sufficiently high to permit transcription on addition of hydrocortisone. Such an explanation (in this case, high levels of C/EBP) has been proposed for the inability of IL-6 to regulate the expression of the α_1-acid glycoprotein gene in the rat hepatoma HTC cell line [18]. However, the 10 h lag phase before the glucocorticoid induction of α_2-macroglobulin in G4.2.3 cells suggests a requirement for the synthesis of additional factors. This requirement can be partially overcome by IL-6 which reduces the lag phase to 4 h. The level of expression of IL-6 receptors in G4.2.3 cells is presently unknown. Glucocorticoids induce the synthesis of IL-6 receptors in some hepatoma cell lines [18] and consequently may induce responsiveness to IL-6 in a previously unresponsive cell.

Other cytokines, e.g. IL-1 and TNFα, are known to affect the synthesis of α_2-macroglobulin, although the response varies depending on the cell lines [19]. The effects of IL-1 may be mediated through IL-6. IL-1 induces the synthesis of IL-6 in several types of cell [6]. The ability of TGFβ to regulate the expression of α_2-macroglobulin is less well documented. In G4.2.3 cells, TGFβ has no effect on the synthesis of fibronectin but increases the synthesis of plasminogen activator inhibitor-1 (PAI-1) 6-fold (unpublished data).

The rat mammary myoepithelial cell line, G4.2.3, thus appears to represent a useful system for studies on the regulation of α_2-macroglobulin synthesis in a non-hepatic cell and on the control of myoepithelial differentiation during pregnancy.

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REFERENCES

[1] Baumann, H. (1989) In Vitro Cell. Dev. Biol. 25, 115–126.
[2] White, R., Habicht, G.S., Godfrey, H.P., Janoff, A., Barton, E. and Fox, C. (1981) J. Lab. Clin. Med. 97, 718–729.
[3] Budek, W., Danailov, M.L., Bunning, P. and Afting, E.G. (1986) Biochem. Biophys. Res. Commun. 134, 57–63.
[4] Gunter, U., Strauss, S., Jonas, U., Weidemann, A., Beyreuther, K., Volk, B., Berger, M. and Bauer, J. (1991) FEBS Lett. 282, 127–131.
[5] Warburton, M.J., Coles, B., Dundas, S.R., Gusterson, B.A. and O'Hare, M.J. (1993) Eur. J. Biochem. 214, 803–809.
[6] Heinrich, P.C., Castell, J.V. and Andus, T. (1990) Biochem. J. 265, 621–636.

[7] Baumann, H., Jahreis, G.P. and Morella, K.K. (1990) J. Biol. Chem. 265, 22275–22281.
[8] Kunz, D.R., Zimmermann, R., Heisig, M. and Heinrich, P.C. (1989) Nucleic Acids Res. 17, 1121–1138.
[9] Hocke, G.M., Barry, D. and Fey, G.H. (1992) Mol. Cell. Biol. 12, 2282–2294.
[10] Hattori, M., Abraham, L.J., Northemann, W. and Fey, G.H. (1990) Proc. Natl. Acad. Sci. USA 87, 2364–2368.
[11] Northemann, W., Shiels, B.R., Braciak, T.A., Hanson, R.W., Heinrich, P.C. and Fey, G.H. (1988) Biochemistry 27, 9194–9203.
[12] Dundas, S.R., Ormerod, M.G., Gusterson, B.A. and O'Hare, M.J. (1991) J. Cell Sci. 100, 459–471.
[13] Jat, P.S. and Sharp, P.A. (1989) Mol. Cell. Biol. 9, 1672–1681.
[14] Warburton, M.J., Mitchell, D., Ormerod, E.J. and Rudland, P.S. (1982) J. Histochem. Cytochem. 30, 667–676.
[15] Dennis, P.A., Saksela, O., Harpel, P. and Rifkin, D.B. (1989) J. Biol. Chem. 264, 7210–7216.
[16] Silberstein, G.B., Flanders, K.C., Roberts, A.B. and Daniel, C.W. (1992) Dev. Biol. 152, 354–362.
[17] Baumann, H. and Gauldie, J. (1990) Mol. Biol. Med. 7, 147–159.
[18] Baumann, H., Morella, K.K. and Campos, S.P. (1993) J. Biol. Chem. 268, 10495–10500.
[19] Andus, T., Geiger, T., Hirano, T., Kishimoto, T., Tran-Thi, T.-H., Decker, K. and Heinrich, P.C. (1988) Eur. J. Biochem. 173, 287–293.