Glucocorticoids induce the expression of the uteroglobin gene in rabbit foetal lung explants cultured *in vitro*

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In 27-day-old rabbit foetal lung explants cultured *in vitro*, the synthesis of the protein uteroglobin decreased progressively during several days of culture. Addition of glucocorticoids to the medium progressively induced the synthesis of uteroglobin in a dose-dependent manner without affecting the synthesis of total proteins. The glucocorticoid-mediated induction of uteroglobin appears mainly due to increased amounts of uteroglobin mRNA and seems to be independent of simultaneous cell proliferation, suggesting a glucocorticoid-triggered differentiation of pre-existing cells. The results suggest a major role of glucocorticoids in the developmental regulation of the uteroglobin gene in the lung.

The late differentiation of foetal lung is under the control of hormones, particularly glucocorticoids (Farrell, 1977). This hormonal control has been extensively studied both *in vivo* and *in vitro*, in particular regarding the metabolism of lipids involved in the synthesis of surfactants. However, the study of the hormonal factors controlling the developmental changes of a single and well-known gene in the lung could throw more light on the mechanisms of differentiation of this organ. Furthermore it is possible that this gene might serve as a marker of lung development.

In the lung of the rabbit and other related species exists a small secretory protein that was first discovered in rabbit uterus and hence called 'uteroglobin' (Beier, 1968). The amount of synthesis of uteroglobin in foetal lung is very low, but it increases rapidly towards the end of gestation and after birth (Lombardero & Nieto, 1981). This increase might, like the other changes occurring in foetal-lung differentiation, be induced by hormonal factors. We have therefore studied the hormonal control of the developmental changes in the expression of uteroglobin, a gene whose structure is now well known (Bailly *et al.*, 1983; Suske *et al.*, 1983). To carry out this study we used a tissue-culture system, a technique that has been widely used in studies of lung development (Ekelund *et al.*, 1975; Snyder *et al.*, 1981; Torday, 1980) and which provides a means of distinguish-

ing between direct hormonal effects and those secondary to interaction of hormones with other target organs.

Materials and methods

Pregnant New Zealand White rabbits (approx. 3 kg) were killed at 27–28 days after mating, and foetuses were aseptically obtained by uterotomy.

Culture of explants

Lungs from several foetuses were cut into small pieces (1–2 mm³), which were combined, and portions (20–30 mg) were placed in 35 mm-diameter tissue-culture dishes containing 1 ml of Dulbecco's minimal essential medium (Gibco) with 10% (v/v) foetal-calf serum. Explants were cultured in a tissue-culture incubator. The medium was changed every 48 h, centrifuged and saved for uteroglobin determination.

Radioimmunoassay for uteroglobin

The procedure was carried out as previously described (Nieto & Beato, 1980). Before the assay, culture medium was treated for 10 min at 25°C with an excess of *Staphylococcus aureus* (Pansorbin; Calbiochem–Behring), to absorb the serum immunoglobulins. For assays of uteroglobin in explants, the tissue was homogenized and portions of the homogenate were taken for radioimmunoassay and for determination of tissue protein.

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Metabolic labelling of explants

Labelling of the explants with [^{35}S]methionine and immunoprecipitation of newly synthesized uteroglobin were carried out as described previously (Lombardero & Nieto, 1981). Immunoprecipitates were analysed by sodium dodecyl sulphate/polyacrylamide-slab-gel electrophoresis (López de Haro & Nieto, 1983) and the gels were dried and autoradiographed.

Determination of uteroglobin mRNA

Poly(A)-containing RNA was prepared from explants by phenol treatment and oligo(dT)-cellulose chromatography (Lombardero & Nieto, 1981). The relative amounts of uteroglobin mRNA were determined by dot-blot hybridization to cloned plasmid-pUG10 uteroglobin complementary DNA (López de Haro *et al.*, 1984). Increasing amounts of poly(A)-containing RNA from explants were denatured with glyoxal and immobilized on nitrocellulose filters as previously described (Thomas, 1980). Filters were hybridized to the labelled pUG10 probe as described (López de Haro *et al.*, 1984). After drying, the filters were counted for radioactivity, which was plotted against the amount of mRNA applied. The

relative amounts of uteroglobin mRNA were determined from the slopes of the straight lines obtained.

Results and discussion

Fig. 1(a) shows the uteroglobin produced by explants during periods of 2 days (i.e. from 0 to 2 days), from 2–4 days, and so on, corresponding to the intervals between the changes of media. It was observed that, in the controls cultured in the absence of cortisol, the amount of uteroglobin decreased progressively with time. In contrast, explants incubated in the presence of $1\text{ }\mu\text{M}$ -cortisol produced increasing amounts of uteroglobin throughout the culture period, the amount of the protein being about 6-fold greater than in the controls after 6 days. In Fig. 1(b) the same data are replotted as the total or cumulative amount of uteroglobin produced by the explants at the indicated times. The initial concentration of uteroglobin in the explants before incubation is also shown. A net synthesis of uteroglobin during the culture period is apparent, since in the presence of cortisol the total amount of uteroglobin per mg of tissue was, at day 6, about 17-fold that found at

Fig. 1. Kinetics of uteroglobin synthesis by foetal lung explants

Cultured explants, together with their corresponding media, were taken at 2-day intervals for uteroglobin determination. (a) Uteroglobin produced during the 2-day periods preceding the indicated times. (b) Total uteroglobin produced at the indicated times. ●, Control; ▲, cultured with $1\text{ }\mu\text{M}$ -cortisol; □, starting tissue. The inset shows the autoradiography of the immunoprecipitated newly synthesized uteroglobin analysed by gel electrophoresis: 1, control; 2, cortisol-treated explants, both cultured for 4 days. The arrow indicates the position of the uteroglobin marker.

day 0. Pulse-labelling of explants with [^{35}S]-methionine, followed by gel electrophoresis of newly synthesized uteroglobin, confirmed the induction of uteroglobin (Fig. 1, inset). The progressive induction of uteroglobin synthesis found in foetal explants is similar to that observed *in vivo* in rabbits of similar chronological age (Lombardero & Nieto, 1981). On the other hand, the synthesis of total protein was identical in both groups of explants (5.1×10^6 and 5×10^6 c.p.m./mg of tissue protein for control and cortisol-treated explants respectively). Thus decreased synthesis of uteroglobin in the absence of cortisol does not appear to be due to a decrease in overall synthesis of proteins in the explants cultured without hormone. It should be noted that foetal lung explants, either controls or cultured with cortisol, actively secreted uteroglobin into the medium, 80–90% of the total uteroglobin being in the medium after 3 days of culture (results not shown).

Fig. 2 shows the dose-response curve for the effects of the glucocorticoids cortisol and dexamethasone on the induction of uteroglobin in foetal lung explants. Cortisol had an optimum effect at about $10 \mu\text{M}$, whereas dexamethasone was shown to be effective at doses 10–100-fold lower, which is in accordance with the relative potencies of these glucocorticoids in other experimental systems (Samuels & Tomkins, 1970; Baxter & Ivarie, 1978). Progesterone or oestradiol (both at $1 \mu\text{M}$) or triiodothyronine (10 nM) were without effect on uteroglobin synthesis.

The amounts of both uteroglobin mRNA and uteroglobin synthesis were measured in explants cultured with or without glucocorticoids to ascertain whether the induction of the protein was associated with increased levels of its mRNA. The results, shown in Table 1, indicated that these hormones did produce important increases in the amounts of uteroglobin mRNA. Increased amounts of translatable uteroglobin mRNA were

also observed *in vivo* in rabbit lung during the perinatal period (Lombardero & Nieto, 1981).

Although 10% (v/v) foetal-calf serum was routinely used in the incubation medium, we observed a similar effect of glucocorticoids on uteroglobin induction in foetal lung explants cultured in medium alone. Hence the above-mentioned glucocorticoid effect was independent of serum cofactors. On the other hand, this effect appears to occur through a differentiation of pre-existing cells without necessity of cellular division, since the same induction of uteroglobin was obtained in the presence of 1 mM -hydroxyurea (to inhibit DNA synthesis; M. S. López de Haro,

Fig. 2. Dose-response curves for the effect of glucocorticoid on uteroglobin synthesis

Explants were cultured for 4 days in the presence of the indicated concentrations (c) of either cortisol (○) or dexamethasone (●). The uteroglobin produced during the last 2-day period (media plus tissue) was determined.

Table 1. Effect of glucocorticoids on uteroglobin production and amounts of uteroglobin mRNA in foetal lung explants. Explants were cultured for 5 days and the uteroglobin produced during the last 2-day period (medium plus tissue) was determined. Total mRNA was also prepared from the explants, and the amounts of uteroglobin mRNA were determined by hybridization to labelled uteroglobin complementary DNA as described in the Materials and method section.

Expt.	Treatment	Uteroglobin ($\mu\text{g/g}$ of tissue)	Uteroglobin mRNA* (c.p.m. hybridized/ μg of total mRNA)
1	None	1.7	45
	Dexamethasone (100 nM)	10.2	215
2	None	4.8	66
	Dexamethasone (100 nM)	43.6	511
3	None	3.8	43
	Dexamethasone (100 nM)	39.7	403

* Determined with radioactive probes of different specific activities.

unpublished work). It should be also mentioned that, in our 'in vitro' system, cortisol also produced other effects, such as an 50% increase in the amount of total phospholipids and histological evidence of enhanced cellular secretion (results not shown). These effects appear to be typical of the action of glucocorticoids in the late stages of lung development (Snyder *et al.*, 1981; Ekelund *et al.*, 1975; Rooney *et al.*, 1976).

In the present study we have used lungs from 27-day-old fetuses, since uteroglobin becomes detectable in rabbit foetal lung between 25 and 29 days after mating (Daniel & Milazzo, 1976), and maximal increases in the synthesis of this protein occur *in vivo* from this age onwards (Lombardero & Nieto, 1981). As a whole, the above results suggest that glucocorticoids play a major role in the developmental induction of uteroglobin in lung and that the uteroglobin gene is also 'turned on' at the late stage of development as part of the general effect of glucocorticoids in foetal lung. Uteroglobin might be useful as a marker of the maturation effect of glucocorticoids in the lung of a mammalian species.

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