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Cell swelling increased the α2-macroglobulin gene expression in cultured rat hepatocytes

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Abstract The effect of cell swelling on the expression of the α2-macroglobulin (α2M) gene was studied in hepatocytes in culture. Hypoosmolarity induced an increase (3-fold increase) in the level of α2M mRNA through a corresponding stimulation of the rate of transcription of the α2M gene. The addition of raffinose (100 mM) corrected the effect of hypoosmolarity at both mRNA and transcriptional level, demonstrating that cell swelling per se was responsible for the observed effect on the expression of the α2M gene. Moreover, the effect of cell swelling was additive to that of interleukin 6, a major mediator of the acute-phase response.

Key words: Hydration state; Swelling; Hepatocyte; α2-Macroglobulin; Interleukin 6

1. Introduction

α2-Macroglobulin (α2M), which is mainly synthesized in the parenchymal cells of the liver, is the major positive acute-phase protein in rats in response to infections [1–3]. The expression of the α2M gene is mainly under the control of interleukin 6 (IL-6) alone or in combination with glucocorticoids [4]. IL-6 and two other cytokines, namely interleukin 1 and tumor necrosis factor-α, are regarded as the major mediators of the acute-phase response. However, Qian and Brosnan [5] recently reported that administration of *Escherichia coli* endotoxin to rat increased hepatocyte volume in vivo. Moreover, changes in cell volume have been reported to modulate the mRNA level of various genes in rat liver [6], and we specified that changes in cell volume might act at a transcriptional level [7]. Taken together, all these results suggested that changes in cell volume might play a role in the induction of acute-phase genes.

The aim of this work was (i) to study whether changes in cell volume may modulate the expression of the α2M gene and (ii) to specify if the presence of IL-6 was required.

2. Materials and methods

2.1. Materials

Dexamethasone was purchased from Sigma and recombinant human interleukin 6 (rh IL-6) from Boehringer. Hybond-N membranes, multiprime DNA-labeling system, [α-^{32}P]dCTP (specific radioactivity, 3000 Ci/mmol), [α-^{32}P]UTP (specific radioactivity, 800 Ci/mmol) and Hyperfilm-MP were from Amersham. Fetal calf serum was from Dutscher (Brumath, France). The probes used were an insert of rat α2M cDNA provided by Dr G. Fey [8] and an insert of 18S rRNA provided by Dr L. Hendriks [9].

2.2. Primary culture of hepatocytes

Isolated hepatocytes from 24-h starved male adult Wistar rats (200–220 g) were prepared as described [10]. Hepatocytes were suspended in the culture medium (199 medium/MEM; 25%:75%) containing 10% fetal calf serum. The cells were seeded in 100-mm-diameter plastic dishes precoated with 400 pg rat tail (type I) collagen, and cultured at 37°C under 5% CO_2 in air. After an attachment period of 4 h, the medium was replaced by fresh culture medium deprived of glutamine containing 5% fetal calf serum and 10^{-7} M dexamethasone (control cells). Hypoosmotic and hyperosmotic media were obtained by decreasing (−50 mM) or increasing (+20 mM) the NaCl concentration of the buffer, respectively. For RNA analysis and nuclear run-on assays, the cells were harvested, frozen in liquid nitrogen and stored at −80°C.

2.3. Incubation of hepatocytes

Hepatocytes (50–70 mg wet wt/ml) were shaken (165 strokes/min) in stoppered scintillation vials at 37°C for the indicated times. The standard incubation medium was a Krebs-Henseleit bicarbonate buffer at pH 7.4 containing 10^{-7} M dexamethasone. All media were in equilibrium with a gas phase of O_2/CO_2 (19:1).

2.4. Extraction and analysis of cellular RNA

Isolation of total RNA was performed by a guanidium-thiocyanate procedure [11]. RNA was separated on 1.5% agarose/formaldehyde gels and transferred to nylon membranes for Northern hybridization. The membranes were hybridized with a random-oligonucleotide-primed ^{32}P-labeled insert as described [12]. Filters were washed and exposed to Hyperfilm at −80°C with intensifying screens. Relative densities of the hybridization signals were quantified by scanning the films with a Biocom densitometer. To correct for differences in RNA loading, all the results were expressed as the ratio of the scanned values for α2M mRNA vs. those for 18S rRNA (relative level).

2.5. Nuclear run-on transcription assay

The preparation of nuclei and the RNA-polymerase elongation reaction were performed essentially as described [13]. For transcription, 9×10^6 nuclei were incubated for 30 min at 28°C in 200 μl 15% glycerol, 50 mM HEPES pH 8.0, 150 mM KCl, 1 mM dithiothreitol, 2.5 mM magnesium acetate, 1 mM $MnCl_2$, 0.5 mM EDTA, 4 mM creatine phosphate, 15 U/ml creatine kinase, 1 mM spermidine, 0.5 mM each of ATP, CTP and GTP, and 500 U/ml RNasin, in the presence of 100 μCi of [α-32P]UTP (800 Ci/mmol). DNAse I (40 U) was added and the samples were incubated at 37°C for 15 min. The labeled RNAs were extracted essentially as described [11]. Denatured plasmids (5 μg) were spotted onto cellulose by means of a slot-blot apparatus. All the DNA-bearing filters were prehybridized, then hybridized with labeled RNA (5×10^6 cpm) at 42°C for 3 days. Hybridization was carried out with three plasmids: α2M; pBR322 as a control for non-specific binding of labeled RNA; and the 18S rRNA probe as the internal control. Filters were washed and exposed to Hyperfilm at −80°C with intensifying screens. The relative amount of labeled nuclear transcripts hybridized to plasmids bound to nitrocellulose was determined by densitometric scanning of autoradiograms. The values obtained for the α2M signal were corrected by means of the 18S values.

2.6. Determination of cell volume

Cell volume was measured as described by Baquet et al. [14].

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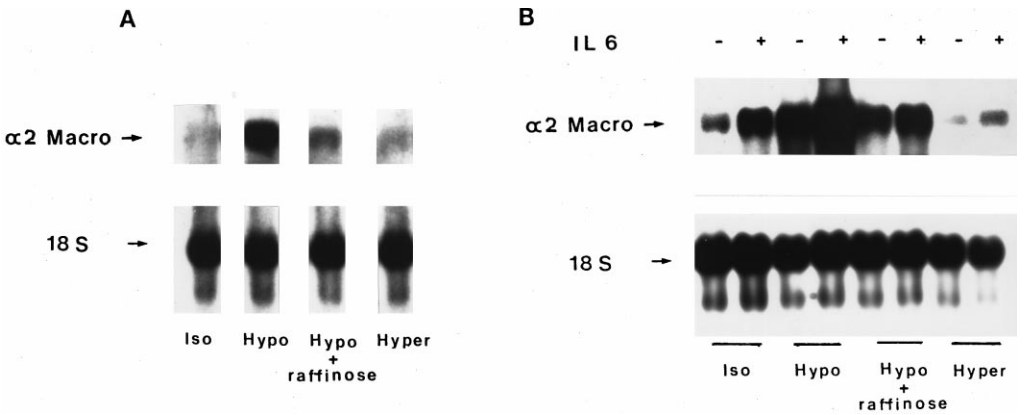

Fig. 1. Influence of anisotonicity on the level of α2M mRNA. Hepatocytes were cultured for 15 h in isoosmotic medium (Iso) or in different osmotic conditions (Hypo, Hyper) in the presence or in the absence of IL-6 (100 U/ml). 100 mM raffinose was added to hypoosmotic medium to restore isoosmotic condition. Total RNA was extracted and 25-μg aliquots analyzed by Northern blot. They were probed successively with the α2M and the 18S cDNAs. The figure shows representative autoradiograms. A: Anisotonicity alone. B: Influence of IL-6 in different osmotic conditions.

2.7. Expression of the results

The results are expressed as means ± S.E.M. for the observations on the indicated number (*n*) of different cell preparations. Statistical significance of differences was calculated by the Student's *t*-test for paired data.

3. Results and discussion

3.1. Cell swelling increased the level of α2M mRNA

Hepatocytes were cultured for 15 h in iso- and hypoosmotic media. Culture of hepatocytes in hypoosmotic condition induced a 3-fold increase in the level of α2M mRNA (1, Iso; 2.97 ± 0.67*, Hypo; $n = 6$; $P < 0.05$) (Fig. 1A). Moreover, the effect of hypoosmolarity on the level of α2M mRNA appeared significantly as soon as 5 h of culture (not shown). The addition of IL-6 (100 U/ml) to the isoosmotic medium induced an increase in the level of the α2M mRNA, as expected (1, Iso; 3.24 ± 0.51*, +IL-6; $n = 3$; $P < 0.05$) (Fig. 1B). Interestingly, the effect of hypoosmolarity was of the same order of magnitude of that of IL-6. These results strongly suggested that cell swelling might be an inducer of the α2M gene. However, the hypoosmotic medium was obtained by decreasing the NaCl concentration. Thus, an effect of the change in the NaCl concentration could not be excluded to explain the observed modulation of the level of α2M mRNA. To test the effect of osmolarity by itself, we used raffinose to correct hypoosmolarity in the medium. The addition of raffinose (100 mM) to hypoosmotic medium blocked the stimulatory effect of hypoosmolarity on the level of α2M mRNA (2.97 ± 0.67, Hypo; 1.33 ± 0.48*, Hypo+raffinose; $n = 6$; $P < 0.05$) (Fig. 1A). This demonstrated that cell swelling per se might stimulate the expression of the α2M gene. However, we were unable to detect a significant decrease in the level of α2M mRNA in cells cultured in hyperosmotic medium, which is known to induce cell shrinkage. This might be due to the very low expression of the gene in control (isoosmotic) conditions. We therefore tested the influence of the modulation of cell volume in cells cultured in the presence of IL-6.

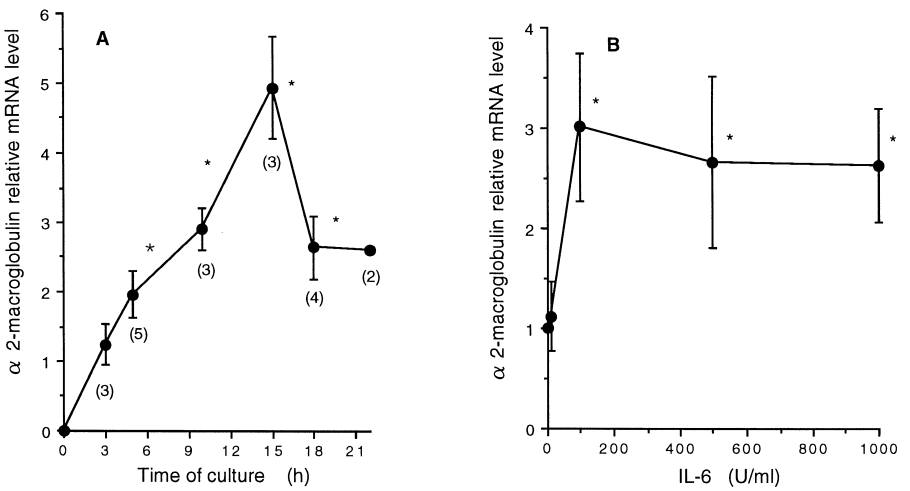

Fig. 2. Influence of IL-6 on the level of α2M mRNA. A: Time-course study. Hepatocytes were cultured for various periods of time in the presence of IL-6 (100 U/ml). The results are means ± S.E.M. for the number of independent experiments given in parentheses. *, Significantly different ($P < 0.05$) from the corresponding control values. B: Dose-response study. Hepatocytes were cultured for 15 h in the presence of various concentrations of IL-6. The results are means ± S.E.M. for 6 independent experiments. *, Significantly different from the value obtained in the absence of IL-6.

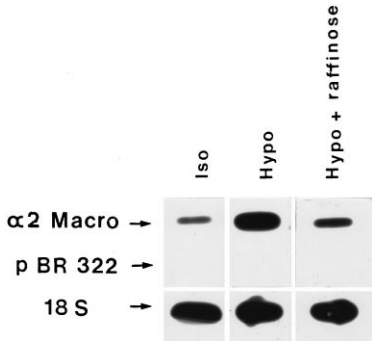

Fig. 3. Influence of anisotonicity on the rate of transcription of the α2M gene. Hepatocytes were cultured for 5 h in different osmotic conditions as described in the legend of Fig. 1. After extraction, nuclei were incubated with [α-^{32}P]UTP, as described. The labelled RNAs were hybridized onto filters containing the α2M cDNA, pBR322 for non-specific binding and the 18S rRNA probe as internal control.

3.2. Cell swelling reinforced the effect of IL-6 on the level of α2M mRNA

Time-course study of the effect of IL-6 addition on the level of α2M mRNA showed that the maximal effect of IL-6 was observed at 15 h in hepatocytes cultured in isoosmotic condition (Fig. 2A). Then, dose-response of the effect of IL-6 on the level of α2M mRNA was studied. Hepatocytes were cultured for 15 h in the presence of various concentrations of IL-6. As shown in Fig. 2B, the maximal effect of IL-6 on the level of α2M mRNA was obtained with 100 U/ml. We therefore tested the influence of hypoosmolarity on the level of α2M mRNA in hepatocytes cultured for 15 h in the presence of 100 U/ml IL-6. Table 1 shows that hypoosmolarity reinforced the effect of IL-6 on the level of α2M mRNA (see also Fig. 1B). Moreover, this table also shows that the addition of raffinose (100 mM) totally blocked the reinforcing effect of hypoosmolarity. Finally, the stimulatory effect of IL-6 decreased in hepatocytes cultured in hyperosmotic condition (Table 1, see also Fig. 1B). These results demonstrate that changes in cell volume modulate the level of α2M mRNA. Moreover, the effect of hypoosmolarity was the same order of magnitude in the presence or in the absence of IL-6. Thus, the effect of cell swelling appeared additive to that of IL-6.

Then experiments were performed to specify if IL-6 per se might induce cell swelling. The experiments were performed using incubated hepatocytes in order to obtain accurate measurement of cell volume. Isolated hepatocytes were incubated in isoosmotic conditions with or without IL-6 (100 U/ml), and the cell volume was measured. Although IL-6 significantly increased the level of α2M mRNA after 3 h of incubation (1, control; 1.60 ± 0.11*, +IL-6; $n = 3$; $P < 0.05$), we were unable to detect any significant change in cell volume after 1 h (100%, control; 99.84 ± 0.36%, +IL-6) or 2 h of incubation (100% control; 100.85 ± 0.61%, +IL-6; $n = 4$). This demonstrated that IL-6 did not involve changes in cell volume to stimulate the expression of the α2M gene.

Table 1
Influence of anisotonicity on the effect of IL-6 on the level of α2M mRNA

Iso	Hypo	Hypo+raffinose	Hyper
1	2.86 ± 0.49*	0.98 ± 0.15	0.38 ± 0.12*

Hepatocytes were cultured for 15 h isoosmotic medium (Iso) or in different osmotic conditions (Hypo, Hyper) with 100 U/ml IL-6. 100 mM raffinose was added to hypoosmotic medium to restore isoosmotic condition. The results are given as means ± S.E.M. for 6 independent experiments. *, Significantly different ($P < 0.05$) from the control (isoosmotic condition) value.

3.3. Cell swelling increased the rate of transcription of the α2M gene

To specify whether a transcriptional mechanism was involved in the effect of hypoosmolarity, run-on transcription assays were performed. Hepatocytes were cultured for 5 h in iso- and hypoosmotic media. As shown in Fig. 3, the rate of transcription of the α2M gene was 3.4-fold higher in hepatocytes cultured in hypoosmotic condition than that measured in isoosmotic condition (1, Iso; 3.41, Hypo; $n = 2$). Moreover, addition of raffinose (100 mM) blocked the stimulatory effect of hypoosmolarity on the rate of transcription of the α2M gene (Fig. 3). This demonstrated that cell swelling increased the expression of the α2M gene at a transcription level.

In conclusion, the data reported here demonstrate that cell swelling increases the α2M mRNA level through a corresponding modulation of gene transcription. This is, to our knowledge, the first report that cell volume regulates the expression of an acute-phase gene. Moreover, our results also show that the effect of cell swelling on the expression of the α2M gene is additive to the effect of IL-6, a major mediator of the acute-phase response. These results strongly suggest that the increase in cell volume might be an additional factor regulating the response of the liver during sepsis.

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