Murine interleukin 1 stimulates α_2-macroglobulin synthesis in rat hepatocyte primary cultures

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In rat hepatocyte primary cultures recombinant interleukin 1 was found to stimulate α_2-macroglobulin synthesis, whereas albumin synthesis was decreased. Although recent experiments gave evidence that a hepatocyte-stimulating factor distinct from interleukin 1 must exist, we conclude that interleukin 1 exerts a direct effect on hepatocytes by inducing acute-phase protein synthesis.

Interleukin 1 *α_2-Macroglobulin* *Rat hepatocyte*

1. INTRODUCTION

The acute-phase proteins are a group of liver-derived plasma proteins which increase in concentration during inflammatory processes, immunologic reactions or tissue injury [1–3]. In the rat these proteins consist mainly of proteinase inhibitors, such as α_2-macroglobulin (α2M), α_1-proteinase inhibitor, α_1-acute-phase globulin, recently identified as cysteine proteinase inhibitor [4,5] and furthermore found to be identical with kininogen [6–9]. The underlying mechanisms for the induction of acute-phase proteins are poorly understood. It has been found that the increased serum levels correspond to elevated mRNA concentrations [10–16]. Recently we have shown by in vitro transcription experiments with isolated nuclei from livers of inflamed rats that α2M gene activity increases 5-fold [17]. Nevertheless, it remains to be clarified which kind of signal(s) reach(es) the hepatocytes and how the signal(s) finally lead(s) to gene activation. In previous studies with rat hepatocyte primary cultures we found that α2M synthesis can be induced by the simultaneous action of glucocorticoids and a Kupffer cell-derived factor (hepatocyte stimulating factor, HSF) [18,19]. Up to now the HSF has not been well characterized. Some authors have claimed interleukin 1 to be identical with this factor [20–22]. On the other hand, evidence has been presented that the HSF is distinct from interleukin 1 [19,23,24]. Since recombinant interleukin 1 became available recently [25,26], we have tested its effect on the induction of α2M synthesis in rat hepatocyte primary cultures. Here we describe that murine interleukin 1 is able to stimulate α2M synthesis in hepatocytes.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[^{35}S]Methionine (>600 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Protosol was obtained from New England Nuclear, Boston, MA, and protein A-Sepharose CL-4B from Pharmacia, Freiburg. RPMI 1640 medium was from Serva, Heidelberg, and fetal calf serum from Boehringer, Mannheim. Recombinant mouse interleukin 1 was a generous gift from Dr P.T. Lomedico, Roche Research Center, Nutley, NJ, and Dr S.B. Mizel, Pennsylvania, PA.

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2.2. *Preparation of rat hepatocyte monclayers*

Suspensions of rat hepatocytes were prepared as described by Tran-Thi et al. [27]. The hepatocyte primary cultures consisted of 97% hepatocytes. Before each experiment the cultures were routinely controlled by microscopic examination. Labeling of hepatocytes was carried out as outlined [28–30]. The preparation of antisera against rat α2M and albumin and the immunoprecipitations have been described [31,32]. For the quantification of the radioactivity incorporated into α2M the respective bands identified by fluorography [33] were cut from the SDS-PAGE gels [34], solubilized with protosol/water (9:1, v/v) at 45°C overnight and counted in a liquid scintillation spectrometer.

2.3. *Interleukin 1 assay*

Interleukin 1 activity was measured in the co-stimulation assay with C3H/HeJ thymocytes as described [35]. The recombinant interleukin 1 was pure and endotoxin-free [25]. The interleukin 1 preparation was in 5 M guanidine-HCl and contained 2×10^6 units per ml.

3. RESULTS

To answer the question as to whether interleukin 1 is capable of inducing acute-phase protein synthesis in hepatocytes, recombinant interleukin 1 was added at different concentrations to rat hepatocyte primary cultures, and the synthesis and secretion of α2M and albumin were measured. Fig.1 shows that interleukin 1 leads to a dose-dependent induction of α2M synthesis. A corresponding increase in α2M is found in the hepatocyte medium (B). Albumin, on the other hand, decreases upon interleukin 1 addition both in cells and media as expected for this negative acute-phase protein.

To demonstrate that all the radioactivity in the α2M bands of fig.1 is associated with α2M, we added unlabeled rat α2M to the cell homogenate of [^{35}S]methionine-labeled hepatocytes before the immunoprecipitation. It is shown in fig.2 that the radioactive band with an apparent M_r of 182000 disappears upon addition of unlabeled α2M.

For further quantification the α2M and albumin bands were excised from the gels and their radioactivity was determined. The data are given in table 1 and represent the mean of 3 experiments. It

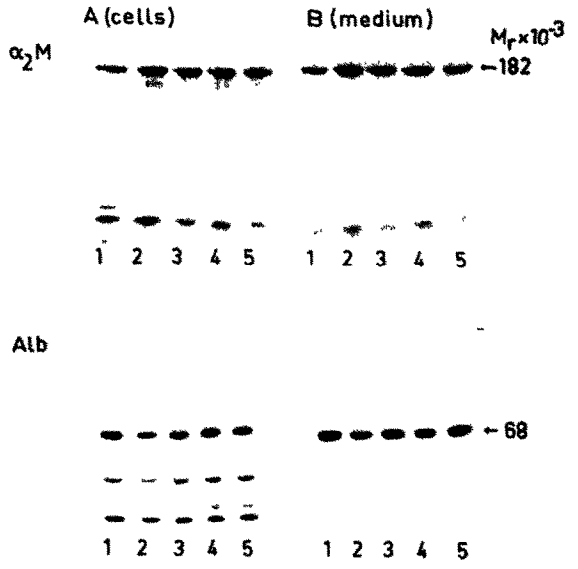

Fig.1. Effect of murine interleukin 1 on α_2-macroglobulin and albumin synthesis in rat hepatocytes. Hepatocyte primary cultures (3.9×10^6 cells/dish) were incubated for 12 h in the presence of 10^{-9} M dexamethasone in a total volume of 3 ml containing different amounts of interleukin 1. The hepatocyte medium consisted of 1 ml Waymouth medium and 2 ml RPMI 1640 medium. Interleukin 1 (2×10^6 units per ml 5 M guanidine-HCl) was diluted with 5 M guanidine-HCl to give 1:10, 1:100, and 1:1000 dilutions. The interleukin 1 stock solutions were further diluted 3000-fold with RPMI medium, extensively dialyzed against RPMI medium and then added to the hepatocyte primary cultures. Lanes: 1, no interleukin 1; 2, 1:3000 dilution of interleukin 1; 3, 1:30000; 4, 1:300000; 5, $1:3\times10^6$. Incubation was carried out at 37°C for 12 h, the media were removed and replaced by 3 ml Waymouth medium without methionine. 25 μCi [^{35}S]methionine were added to each dish. After a labeling period of 90 min, cells (A) and media (B) were separated and α2M and albumin were immunoprecipitated. Comparable amounts of total trichloroacetic acid-precipitable radioactivity were used for the immunoprecipitation (2×10^6 cpm in the case of cells and 0.5×10^6 cpm in the case of media).

should be noted that dexamethasone was present in all our hepatocyte primary cultures, since the presence of glucocorticoids is a prerequisite for the synthesis of α2M as shown in [19]. In the experiments presented a concentration of 10^{-9} M dexamethasone was used.

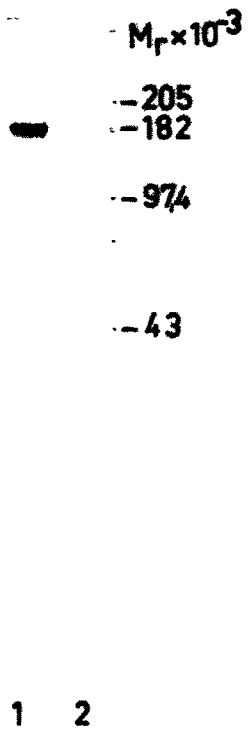

Fig.2. Immunoprecipitation of [^{35}S]methionine-labeled α2M in the absence and presence of unlabeled α2M. Hepatocyte primary cultures (3.9×10^6 cells) were labeled with 25 μCi [^{35}S]methionine for 3 h. A total of 4×10^6 cpm trichloroacetic acid-precipitable radioactivity was used for immunoprecipitation in the absence (lane 1) or presence of 50 μg unlabeled rat α2M (lane 2).

Table 1

Effect of interleukin 1 on the synthesis of α_2-macroglobulin and albumin in rat hepatocytes

Interleukin 1 units per dish	Radioactivity in immunoprecipitated protein (% × 10^2)			
	α_2-Macroglobulin		Albumin	
	Cells	Medium	Cells	Medium
0	5.2	17.4	2.5	47.0
0.07	7.6	22.9	2.3	42.5
6.7	8.3	34.1	2.2	36.8
67	9.1	38.8	1.9	26.9
667	11.5	49.8	1.5	22.7

The radiolabeled α_2-macroglobulin and albumin bands according to fig.1 were excised from the gels and the radioactivity determined. Values given are means of 3 experiments. The data are percentages of total trichloroacetic acid-precipitable radioactivity in hepatocytes and hepatocyte medium

4. DISCUSSION

Recent experiments from our laboratory [19] have shown that supernatants from lipopolysaccharide-stimulated and unstimulated rat Kupffer cells or human monocytes exhibit the same hepatocyte-stimulating activity as measured by α2M induction, although the supernatants from the lipopolysaccharide-stimulated cells contained a 10-times higher interleukin 1 activity. These data suggested that interleukin 1 was not involved to a major extent in hepatocyte stimulation.

Furthermore, Ritchie and Fuller [36], Baumann et al. [23] as well as Koj et al. [24] demonstrated by means of Sephadex chromatography that HSF and interleukin 1 activities in supernatants of human monocytes can be separated. These findings seem to be in contrast to the observations presented here that show that recombinant murine interleukin 1 stimulated α2M synthesis in rat hepatocytes. It seems likely, however, that a HSF activity exists that is different from interleukin 1.

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