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CHANGES IN RAT LIVER mRNA FOR ALPHA-1- ACID-GLYCOPROTEIN, APOLIPOPROTEIN E , APOLIPOPROTEIN B AND BETA-ACTIN AFTER MOUSE RECOMBINANT TUMOR NECROSIS FACTOR INJECTION

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SUMMARY: Hybridization studies using specific cDNA probes have been used to determine the specific mRNA levels for apolipoproteins B and E , α1 acid glycoprotein and β actin in extracts of rat liver . Injection of rats with recombinant mouse tumor necrosis factor had led to a rapid increase in liver mRNA levels for α1 acid glycoprotein (x 12) and for β actin (x 2.5) whereas mRNA levels for Apolipoprotein B and E remained stable over the same period .

Tumor Necrosis Factor (TNF) , also known as Cachectin , has been demonstrated to supress lipoprotein lipase (LPL) expression by adipocytes in vitro [1].This action might account for hypertriglyceridemia occurring during trypanosome induced cachexia [2] or , in critically ill patients after a bad accident [3] and in

ABBREVIATIONS

TNF :Tumor necrosis factor . rm TNF : murine recombinant TNF LPL : lipoprotein lipase . HDL : high density lipoprotein . Apo E and Apo B : Apolipoprotein E and B . α1GP : Alpha-1-acid-glycoprotein HSF : Hepatocyte stimulating factor.IFNβ2 : Interferon beta 2 IL6 : Interleukin 6 . IL1 ; Interleukin 1 .

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patients with moderate burns [4]. TNF is released by a wide range of cells stimulated by factors or conditions well known to induce acute inflammation [for review , see ref 5]. We have recently shown that the injection of murine recombinant TNF into rats induced a typical hepatic acute phase response [6] .

Lipoproteins of the high density class (HDL) contain an acute phase negative protein ,apolipoprotein A IV , in the rat [7] . In addition , critically ill patients also exhibit a decrease in plasma cholesterol and apolipoprotein B [3] . Furthermore , in humans , serum amyloïd A (Apo SAA), a positive acute phase protein is an apoprotein in the HDL3.class of lipoproteins .

The changes in the rate of synthesis of specific plasma proteins by the liver during the acute phase response have been closely correlated with the changes in the corresponding mRNA levels [9-11] . In the present study , we have examined the modifications in the liver mRNA levels for the two main apolipoproteins involved in liver secretion and clearance of triglyceride rich lipoproteins , i.e. apolipoproteins E and B, under TNF stimulation . Furthermore , the liver content of ß Actin mRNA was determined under the same conditions and the hepatic acute phase response was checked by the following of α1 GP m RNA .

MATERIALS AND METHODS

Animals. Pathogen free 4-5 weeks old WAG male rats weighing 70-80 g (Charle Rivers France) were injected subcutaneously with 6 µg of recombinant murine TNF . TNF having a specific activity of 1.6 10^7U/mg was kindly given by Dr W.FIERS (Ghent , Belgium).Blood samples and liver were removed under ether anesthesia.

cDNA Probes. Rat apoE cDNA was isolated by immunoscreening of a rat liver cDNA λGT11 library as described [12] . Rat apo B cDNA was isolated by sreening of the same library using a human apo B cDNA probe [13] . Rat α1 GP and Human ß Actin cDNAs were respectively given by Dr Taylor and Dr M Buckingham . The labelled cDNA probes were prepared by nick translation with [alpha 32 P]-dCTP with a specific activity about 10^8 cpm/µg [14] .

RNA preparation .Livers were homogenized in 4M guanidium thiocyanate solution (pH 7.00) containing 0.1% (w/v) N-Lauryl

sarcosine, 25mM trisodium citrate and 0.1 M mercaptoethanol . RNAs were extracted according to Cox [15]. The integrity of RNA preparations was assayed by electrophoresis and visualization of 18S and 28S ribosomal RNAs.using ethidium bromide . The 28S/18S ratio was always close to 2 without any detectable low molecular weight forms .

Northern blot analysis .Total RNA was denatured for 15 min at 55°C in 10 mM sodium phosphate buffer (pH 7.00) containing 2.2 M formaldehyde and 50% formamide, then applied to a 0.8% agarose gel in the same buffer without formamide and separated by electrophoresis at 100 V for 4 hr [16]. Transfer was performed for at least 12 h onto Hybond nylon filters in 20 SSC [17] . The filters were then baked and hybridized. Hybridization was performed at 42°c in 6 x SSC containing 5x Denhardt's solution , 0.1% sodium dodecyl sulfate and 50% formamide in the presence of [alpha ^{32}P]-cDNA probes ($2x10^6$cpm /ml).The intensity of mRNA bands was measured by quantitative scanning densitometry of the autoradiograms of the filters.

Quantitation of mRNA by dot-blot analysis Total RNA was diluted in 10 mM PBS (pH 7.4) and its concentration was checked by absorbance determination at 260 nm . After denaturation in 2.2 M formaldehyde for 10 min at 60°C , RNA samples and three subsequent 1/2 dilutions were dotted onto nitrocellulose filters . Hybridization was performed as described above . After autoradiographic exposure the dots were cut out and counted by liquid scintillation . Radioactivity was linear with RNA amounts dotted onto the filters.

Quantitation of plasma α1 acid glycoprotein was performed as described previously [6] .

RESULTS

Injection of recombinant murine TNF into rats induced both an increase in plasma level of α1 acid-glycoprotein which continued to rise after 24 h (Fig-1) and an increase in its specific mRNA level among the total liver RNAs as evidenced by dot-blot experiments (Fig-2) and Northern blotting (Fig-3) . This is in accordance with our previous results showing a rise in the rat plasma levels of haptoglobin ,α2 macroglobulin ,α1 acid glycoprotein and fibrinogen , peaking 24 h after rm TNF injection [6] . The effect

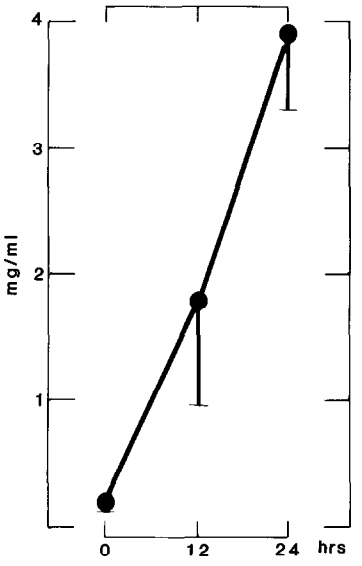

FIGURE 1
Evolution of the plasmatic level of α1GP during the time course of TNF induced liver acute phase response . α1GP level was determined by electroimmunodiffusion of plasma samples diluted 20 fold , in agarose gel containing 0.5% (v/v) of monospecific immunserum anti α1GP .

of TNF on the induction of the major acute phase proteins might be related to an indirect effect through the liberation of hepatocyte stimulating factors ,i.e. HSF I and HSF II [18] . Furthermore Gauldie et al. have demonstrated the identity of HSF II with interferon ß 2 (IFN ß2) [19] , also named Interleukin 6 (IL6) [20] . Interleukin 6 ,but neither TNF nor IL1 , induced a rise in the amount of α2 Macroglobulin and cysteine proteinase inhibitor/T kininogen secreted by rat hepatocyte primary culture , as well as a rise in their specific mRNA level [22]. However , uncertainties remain for the induction of α1GP synthesis in rat hepatoma cells by HSF I ,a mediator which is clearly distinct from IFNß2/IL6 [23] . The stimulations of α1GP secretion induced by IL1 , TNF or murine macrophage supernatant in rat hepatocytes are similar [24] . Moreover , a mixture of anti IL1 and anti IL6/IFNβ2 antibodies can completely supress the induction of α1GP secretion by a dialysed supernatant from peripheral blood monocytes in HEP G2 hepatoma cells whereas anti IFNß2 antibodies alone cannot[19]. In such hepatoma cells ,a complete induction of α1GP secretion cannot be obtained witout a cooperative stimulation by IFN β2/IL6 and IL1[23] .

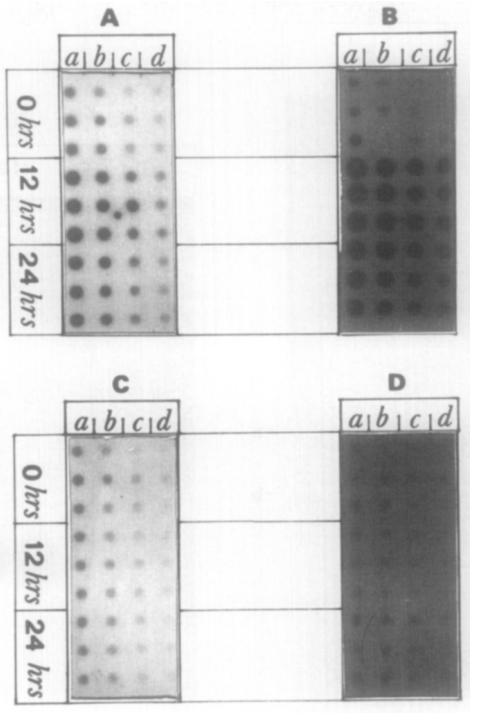

FIGURE 2

Dot blot analysis of rat liver RNA extracts obtained at 0 , 12 , and 24 hours during the time course of TNF induced liver acute phase response. 8µg (a) , 4µg (b) , 2µg (c) and 1µg (d) of each RNA extract were dotted onto nitrocellulose sheet and then hybridized with ^{32}P labelled cDNA probes specific for ß actin (A) , α1 GP (B) , Apo E (C) and Apo B (D).

The Apo-E mRNA levels did not significantly vary after TNF injection (fig-2 and Fig-3B) . This result is in agreement with the statement made by Tu-Guo-Fen et al. that Apo-E mRNA remained at a relatively constant level during experimentally induced inflammation [8] .

The stability of Apo-B mRNA level among total liver RNAs during TNF-induced acute phase response (Fig-2) strengthened the hypothesis that the modifications in VLDL and LDL levels and compositions observed in various inflammatory diseases [2-4] are mainly due to an inhibition of lipoprotein lipase (LPL) expression in adipose tissue [25]. This is strongly supported by the down regulation of LPL gene expression induced by human recombinant TNF in mouse 3T3.L1 adipocytes [26]. Furthermore the

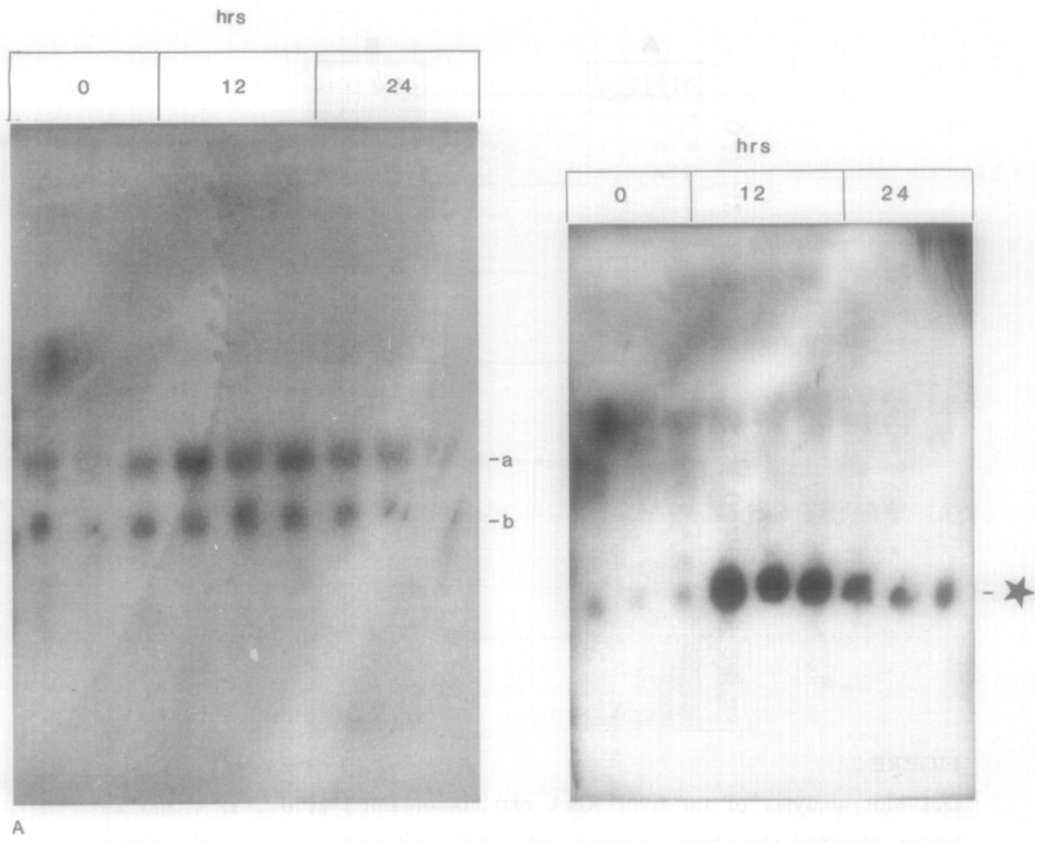

FIGURE 3

Northern blot analysis of rat liver RNA extracts obtained at 0h , 12h and 24h of the TNF induced acute phase response.Each sample , 80 mg of total RNA was layered on agarose gel and electrophoresed. After blotting on a nylon sheet , immobilized RNA were hybridized with ^{32}P labelled cDNA probes . part A: ß actin (a) or ApoE (b). part B : α1GP .

twofold increase in ß actin gene expression in the liver (Fig-2 and Fig-3) is in agreement with the rise of actin synthesis that was observed in mice liver during an acute phase response induced by lipopolysaccharide [27].This might explain the broad modifications in the rate of liver secretion during the inflammatory response . Such modifications fit well with an increase in the hepatic secretion rate of acute phase proteins which was recently described for rabbit CRP [28] .They also might affect VLDL secretion ,since cytochalasin D, a relatively specific inhibitor of actin microfilament , has been demonstrated to interfere with lipoprotein secretion in cultured hepatocytes [29].

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