Tumor necrosis factor-alpha inhibits albumin gene expression in a murine model of cachexia.

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J Clin Invest. 1990;85(1):248-255. https://doi.org/10.1172/JCI114419.

Research Article

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Tumor Necrosis Factor-α Inhibits Albumin Gene Expression in a Murine Model of Cachexia

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Abstract

The mechanisms responsible for decreased serum albumin levels in patients with cachexia-associated infection, inflammation, and cancer are unknown. Since tumor necrosis factor-α (TNFα) is elevated in cachexia-associated diseases, and chronic administration of TNFα induces cachexia in animal models, we assessed the regulation of albumin gene expression by TNFα in vivo. In this animal model of cachexia, Chinese hamster ovary cells transfected with the functional gene for human TNFα were inoculated into nude mice (TNFα mice). TNFα mice became cachectic and manifested decreased serum albumin levels, albumin synthesis, and albumin mRNA levels. However, even before the TNFα mice lost weight, their albumin mRNA steady-state levels were decreased $\approx$ 90%, and in situ hybridization revealed a low level of albumin gene expression throughout the hepatic lobule. The mRNA levels of several other genes were unchanged. Hepatic nuclei from TNFα mice before the onset of weight loss were markedly less active in transcribing the albumin gene than hepatic nuclei from control mice. Therefore, TNFα selectively inhibits the genetic expression of albumin in this model before weight loss. (*J. Clin. Invest.* 1990. 85:248–255.) albumin • cachexia • liver tumor necrosis factor-α, transcription

Introduction

Serum albumin levels are decreased in patients with cachexia-associated infection, inflammation, and cancer, and the reduction in serum albumin levels is used clinically as an indicator of the severity of the chronic disease state (1). Serum albumin levels reflect a complex interaction between the synthesis, volume of distribution, degradation, and losses of this protein. The mechanisms responsible for the hypoalbuminemia of cachexia are unknown. It has been shown that the hypoalbuminemia of chronic inflammatory diseases (2) and of chronic renal failure (3) is at least in part the result of decreased albumin synthesis. If albumin synthesis is decreased in cachexia, then this could be either a nonspecific effect of malnutrition or the specific effect of a humoral factor inhibiting albumin synthesis.

Tumor necrosis factor-α (TNFα),[1] a product of monocytes/macrophages, has been incriminated in the pathogenesis of cachexia (4, 5). The administration of TNFα induces cachexia in animals (6, 7) as well as a negative nitrogen balance in patients (8). In addition, serum levels of TNFα are elevated in some patients with cachexia-associated chronic diseases including cancer (9), parasitic infections (4), and the acquired immunodeficiency syndrome (10). TNFα is a potent modulator of protein synthesis, and many of the severe metabolic abnormalities found in cachexia have been attributed to the effects of TNFα (4, 5). The question of whether TNFα inhibits albumin synthesis in vivo independent of malnutrition and weight loss remains to be determined. Therefore, we evaluated the regulation of albumin gene expression in a murine model of cachexia induced by chronically elevated levels of serum TNFα. We found that TNFα inhibits albumin synthesis at the transcriptional level even before the onset of weight loss.

Methods

Materials

[α-^{32}P] 2′-Deoxycytidine 5′-triphosphate and [α-^{32}P]uridine 5′-triphosphate were from ICN ImmunolBiologicals (Irvine, CA); [^{35}S] cytidine 5′-triphosphate (1,320 Ci/mmol) was from New England Nuclear (Boston, MA); ribonucleotides, deoxyribonucleotides, Klenow fragment of DNA polymerase I, RNase-free bovine serum albumin, dextran sulfate, and ribonuclease A were from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ); agarose, DNase I, RNase TI, and restriction endonucleases were from Bethesda Research Laboratories (Gaithersburg, MD); low melting point agarose and fast green were from Bio-Rad Laboratories (Richmond, CA); guanidine isothiocyanate was from International Biotechnologies, Inc. (New Haven, CT); Biodyne transfer membrane was from Pall Corp. (Glen Cove, NY); nitrocellulose membrane was from Schleicher & Schuell (Keene, NH); 4-morpholinepropanesulfonic acid (MOPS), guanidine HCl, antifoam emulsion, *N*-lauroyl sarcosine, lauroyl sulfate sodium salt, albumin reagent (BCG) and crystal violet were from Sigma Chemical Co. (St. Louis, MO); formamide was from EM Scientific (Gibbstown, NJ); formaldehyde solution was from Mallinckrodt Chemical Co. (Paris, KY); protease K, 1,4-piperazinediethanesulfonic acid (PIPES), and Sephadex G-50 prespun columns were from Boehringer-Mannheim (Indianapolis, IN); agar, tryptone, yeast extract were from Difco Laboratories (Detroit, MI); embedding medium (OCT compound) was from ICN ImmunoBiologicals; rabbit anti-mouse albumin antibody was from Cappel Laboratories (Malvern, PA); biotinylated goat anti-rabbit IgG and the alkaline-phosphatase system were from Vector Laboratories (Burlingame, CA); the monoclonal TNFα immunoassay was from Endogen (Boston, MA); recombinant human TNFα was from AmGen Biologicals (Thousand Oaks, CA); and nude mice were from Simonsen (Gilroy, CA) and Harlan Sprague Dawley (Indianapolis, IN).

Mouse model of cachexia. Chinese hamster ovary cells transfected with either the TNFα gene cloned into a mammalian expression vector (TNFα cells) or with the mammalian expression vector alone (CHO cells, control) were kindly provided by Dr. Oliff (Merk Sharp & Dohme Research Laboratories, West Point, PA) (6). Cells were grown in Dulbecco's modified essential medium supplemented with 10% fetal calf serum, penicillin (50 U/ml) and streptomycin (50 μg/ml). The TNFα cells but not the CHO cells produced TNFα as measured by a

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Received for publication 28 March 1989 and in revised form 5 September 1989.

1. *Abbreviations used in this paper:* MOPS, 4-morpholinepropanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; TNFα, tumor necrosis factor-α.

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Volume 85, January 1990, 248–255

cytolytic assay. 4-wk-old nude mice were injected intramuscularly with either 10^7 CHO cells or 10^7 TNFα cells. Nude mice were housed in a temperature and humidity controlled facility. Animals had free access to food and water and they were killed at 14–21 d (before the onset of weight loss of the TNFα mice) and at 28–35 d postinoculation (after the onset of weight loss). TNFα cells at later passages markedly decrease their production of TNFα (6). When animals were inoculated with these TNFα cells they developed cachexia at ~ 10 wk.

TNFα levels were measured by a biological cytolytic assay, using a modification of the method of Armstrong et al. (11). L929 cells (kindly provided by G. Granger, University of California, Irvine) were plated at 3×10^4 cells per well in 0.1 ml of Eagle's minimal essential media containing 5% fetal calf serum, 2 mM glutamine, and 0.5 μg/ml mitomycin in 96-well tissue culture plates. These plates were incubated at 37°C in 95% air/5% CO_2 for 24 h. Serial dilutions of TNFα samples and standards in media were added to the wells. The plates were incubated for an additional 48 h, drained of media, fixed with methanol, stained with 0.2% crystal violet for 30 min, solubilized with 10% methanol, and the absorbance at 540 nm was measured using a Biotech EL310 plate reader. Results were expressed in units of activity (1 μ = ~ 20 pg). In addition, serum TNFα were determined by an immunoassay using monoclonal antibodies against TNFα, according to the manufacturer's protocol.

Serum albumin concentrations. Serum albumin concentrations were determined by reacting serum or mouse albumin standards with bromocresol green, pH 4.2, at 25°C for 1 min and measuring absorbance at 628 nm (12).

Immunohistochemical staining. At 14–21 d and 28–35 d postinoculation with CHO or TNFα cells, mice livers were fixed in situ by perfusion with 4% paraformaldehyde in PBS via the aorta. The livers were sliced into 3-mm thick sections, postfixed for 2 h at 4°C in the same paraformaldehyde solution. The livers were washed in cold PBS for 10 min, cryoprotected in 30% sucrose PBS for 4 h and embedded and frozen in OCT compound at −70°C. 8-μm cryostat sections were obtained and placed onto slides that had been pretreated with a solution of 100 μg/ml of poly-L-lysine in 10 mM Tris, pH 8.0. Rabbit anti-mouse albumin was purified by affinity chromatography on a Reactigel 6× (Pierce Chemical Co., Rockford, IL) column coupled to mouse albumin according to the manufacturer's procedure. The affinity purified rabbit anti-mouse albumin was used as the primary antibody in conjunction with biotinylated goat anti-rabbit IgG and the ABC-alkaline phosphatase system essentially as recommended by the manufacturer. Blocking was performed with gelatin instead of with serum. Nonspecific alkaline phosphatase reactions were blocked with levamisole and nonspecific antibody reactions assessed by omitting the first antibody. Fast green was used to counterstain the tissue sections.

Northern blotting. Total RNA from individual mouse livers was extracted in guanidine thiocyanate and pelletted by centrifugation of the extract through a cesium chloride cushion. Poly A+ RNA was purified by oligo dT cellulose chromatography. The plasmid pmalb2 (for albumin), pKα1 (for α tubulin), and pMLC3-1 (complement component C3), and pα2M (α2 macroglobulin) were kindly provided by S. Tilgham (13), N. Cowan (14), and G. Fey (15, 16), respectively. The cDNA inserts were purified and radiolabeled with [α-^{32}P] dCTP using the random primer synthesis method (17). 2 μg of poly A+ RNA per lane were electrophoresed in a 1% agarose gel. The gels were transferred to nylon filters and Northern blots were performed as described previously (18). The autoradiograms were quantitated by scanning with a laser densitometer interfaced with an integrator.

In situ hybridization. In situ hybridization was performed by a modification of published methods (19, 20). The albumin cDNA insert of the plasmid palb2 was subcloned into the SP6, T7 transcription vector pGEM blue 3 (Promega Biotec, Madison, WI). Antisense cRNA (complementary to the albumin messenger RNA) and sense RNA probes were generated from the same plasmid using [^{32}S]CTP without additional cold CTP. The radiolabeling reaction was performed according to the manufacturer's protocol with 0.075 nM CTP in a 5-μl reaction volume. The radiolabeled cRNA probe was precipitated with 50 μg of yeast tRNA and then resuspended in 80 μl of 10 mM DTT. The probe was reduced in size by alkaline hydrolysis, ethanol precipitated, and then resuspended in 10 mM DTT 50% formamide.

The cryostat sections described above were postfixed in 4% paraformaldehyde in PBS for 5 min, washed in PBS, and dehydrated through graded ethanol. The sections were rehydrated and deproteinized with protease K 10 μg/ml, 100 mM Tris, pH 8.0, 50 mM EDTA for 20 min. The slides were rinsed in water and then acetylated in 0.25% (vol/vol) acetic anhydride, 0.1 M triethanolamine, pH 8.0 for 10 min. The slides were then washed in 2× SSC for 4 min, dehydrated, and air dried. The hybridization solution consisted of 50% formamide, 300 mM NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA, 100 mM DTT, 5× Denhart's solution, 1 mg/ml yeast tRNA, and 10% dextran sulfate. The [^{32}S]CTP-labeled RNA probe was added to the hybridization solution to give a final concentration of 5×10^5 cpm/slide. The hybridization solution was heated for 3 min at 65° and then 20 μl was added to each slide. A coverslip was applied and sealed with glue. Hybridization was carried out in a humidity chamber at 50°C for 18 h. The coverslips were removed and the slide washed four times in SSC and then incubated in 20 μg/ml RNase and 10 U/ml RNase T1 in 0.5 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA at 37°C for 1 h. The slides were then washed in descending concentrations of SSC containing 1 mM DTT with the most stringent washing being at 55°C in 0.1× SSC. The slides were dehydrated in graded ethanol containing 0.3 M ammonium acetate. After air drying, the slides were dipped in a Kodak NTB2 emulsion containing 0.33 M ammonium acetate. The slides were exposed for 5 d at −20°C, and then developed. The sections were counterstained with hematoxylin and photographed using an IGS filter cube (Nikon).

Nuclear runoff transcription assay. Mouse liver nuclei were prepared by a modification of the procedure of Gorski et al. (21). Livers from two mice were combined and homogenized in 2.4 M sucrose 4:1 (vol of sucrose solution/weight of liver) in a glass Dounce homogenizer with a loose-fitting pestle. The homogenized liver was placed above a cushion consisting of 2.0 M sucrose, 10% glycerol. The nuclei were pelletted by a 130,000 *g* centrifugation at 0°C for 1 h in an SW28 rotor. They were resuspended in the transcription runoff assay buffer, washed once, and then resuspended again in this buffer. Nuclei were frozen at −70°C. The nuclear runoff transcription assay that was developed by Groudine et al. (22) and adapted by Wang and Calame (23) was used as previously described (18). After filters containing the denatured cDNA probes were hybridized with the radiolabeled RNA and washed repeatedly, the radioactivity was determined by liquid scintillation spectroscopy for individual cDNAs. Separate filters containing denatured plasmid pGEM blue 3 were used as negative controls. The transcriptional rate is expressed as the radioactivity incorporated into a specific mRNA that is detected by its hybridization to the denatured cDNA.

Results

2 wk after their inoculation with TNFα cells, mice had serum TNFα concentrations as measured by a biological assay comparable to those reported previously (6). Serum TNFα levels remained elevated in TNFα mice at 4–5 wk after inoculation (Table I). The presence of circulating human TNFα was confirmed with a specific immunoassay utilizing monoclonal anti-human TNFα antibodies (Table I). TNFα was never detected in CHO mice (Table I). The TNFα and CHO mice maintained identical weights for about the first 2–3 wk after inoculation. The TNFα mice then began to progressively lose weight, whereas the CHO mice did not. As previously reported (6), TNFα mice at 4–5 wk after inoculation manifested severe weight loss (Fig. 1). The cachectic TNFα mice had significantly decreased serum albumin levels compared to the CHO control mice (Table II).

Table I. Serum TNFα Levels in TNFα Mice

Experimental conditions*	Serum TNFα (ng/ml) L929 assay‡	ELISA§
TNFα mice (before onset of weight loss)	0.08±0.003	0.3±0.1
TNFα mice (after onset of weight loss)	48±0.5	1.5±0.4
CHO mice	ND	ND

* Nude mice were inoculated with either TNFα-cells (TNFα-mice) or CHO cells (CHO-mice) as described in Methods.
‡,§ Serum TNFα levels were determined by a biological assay (L929) and by an immunoassay (ELISA) as described in Methods. Values are mean±SEM of at least triplicate samples.
ND, none detected.

Table II. Serum Albumin Levels in CHO and TNFα Mice

Experimental conditions*	Serum albumin‡
	g/dl
Experiment I: Precachexic TNFα mice	
CHO mice, 2 wk (n = 5)	1.9±0.1
TNFα mice, 2 wk (n = 6)	1.7±0.1
Experiment II: Cachexic TNFα mice	
CHO mice, 4 wk (n = 4)	2.7±0.2
TNFα mice, 4 wk (n = 5)	2.1±0.1
TNFα mice, 10 wk (n = 4)	1.7±0.1

* Nude mice were inoculated with either CHO cells (CHO-mice) or TNFα cells (TNFα-mice) as described in Methods. The number of weeks designates the time postinoculation of cells.
‡ Determined as described in Methods. $P < 0.05$ for TNFα mice, 2 wk, $P < 0.01$ for TNFα-mice, 4 wk and $P < 0.001$ for TNFα-mice, 10 wk.

Livers from cachectic TNFα and CHO mice were perfused in situ and used for immunohistochemical studies. In the CHO mice, albumin was detected in high abundance diffusely through the hepatic lobule. On the other hand, in the cachectic TNFα mice, the abundance of albumin was greatly depressed (Fig. 2). As controls, sections were incubated in the absence of first antibody, which did not demonstrate any specific staining, or with antimurine Ig as the first antibody, which did not demonstrate any hepatocyte specific staining (data not shown). Northern blot analysis revealed a marked decrease in the steady state levels of albumin mRNA but not of β-actin mRNA in the cachectic TNFα mice compared with the CHO mice (Fig. 3 *A*). The decrease in hepatic albumin protein and mRNA levels in this model of cachexia is comparable to other models of weight loss reported previously by us (24) and others (25, 26).

If TNFα modulates albumin gene expression independently of its effects of malnutrition and weight loss, we would expect TNFα to decrease albumin production before the onset of weight loss. Before the onset of weight loss, TNFα mice had significantly decreased serum albumin levels compared to age-matched CHO control mice (Table II). In addition, immunohistochemically detected albumin was decreased in the hepatocytes of TNFα mice before weight loss (data not shown).

In order to investigate the mechanism by which TNFα inhibits albumin production, expression of the albumin gene was assessed by measuring steady state mRNA levels, cellular localization, and transcriptional activity. Hepatic albumin mRNA content was measured in 10 precachetic TNFα mice and 10 age-matched CHO mice, and a representative Northern blot is shown in Fig. 3 *B*. The albumin mRNA levels of the CHO mice were 100 ± 10.3 (mean ± SEM in relative densitometry units) and of the TNFα mice were 13.3 ± 3.8 ($P < 0.005$). Additional analysis of hepatic mRNA revealed no changes in β-actin, complement C3, or α-tubulin levels in the TNFα mice (Fig. 3 *B*).

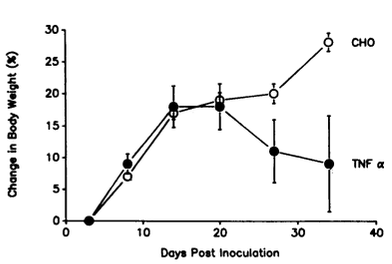

Figure 1. Change in weight of CHO and TNFα mice postinoculation of CHO or TNFα cells. CHO or TNFα cells (10^7) were inoculated into age-matched male nude mice. Each point represents the mean weight change±SEM of at least four mice.

To assess the expression of the albumin gene throughout the hepatic lobules, we performed in situ hybridization using a ^{35}S-albumin antisense cRNA probe. In the CHO mice, abundant albumin mRNA was detected in the liver by in situ hybridization (Fig. 4 *A*). As previously reported (27), highest levels of albumin mRNA were found in the periportal zone (acinar zone 1) with diminished levels peripherally (acinar zone 3). In contrast, the TNFα mice before weight loss had a marked decrease in albumin mRNA levels throughout the hepatic lobule, with loss of the gradient between acinar zones 1 and 3 (Fig. 4 *B*). Minimal background in situ hybridization was observed when a ^{35}S-labeled sense albumin RNA probe was used as a control, therefore, validating the specificity of the antisense probe (Fig. 4, *C* and *D*).

Decreased albumin mRNA levels may reflect either decreased albumin gene transcription or increased albumin mRNA degradation. Transcription runoff assays were performed in order to measure albumin gene expression in hepatic nuclei obtained from TNFα and CHO mice 16 d postinoculation. Albumin gene transcription was decreased by 90% in TNFα murine livers in a representative runoff assay (Fig. 5). This inhibition of albumin gene transcription apparently accounts for the decreased albumin mRNA in the TNFα murine livers. The transcriptional rates of the β-actin gene were unchanged (Fig. 5) suggesting a selective effect of TNFα on albumin gene expression.

Discussion

In this study we use a novel model of cachexia to maintain chronically elevated serum levels of TNFα in mice. The results demonstrate that elevated serum levels of TNFα lead to decreased albumin gene transcription (Fig. 5), decreased steady state levels of albumin mRNA throughout the hepatic lobule (Figs. 3 and 4), decreased albumin production (Fig. 2), and decreased serum albumin levels (Table II). The inhibition of

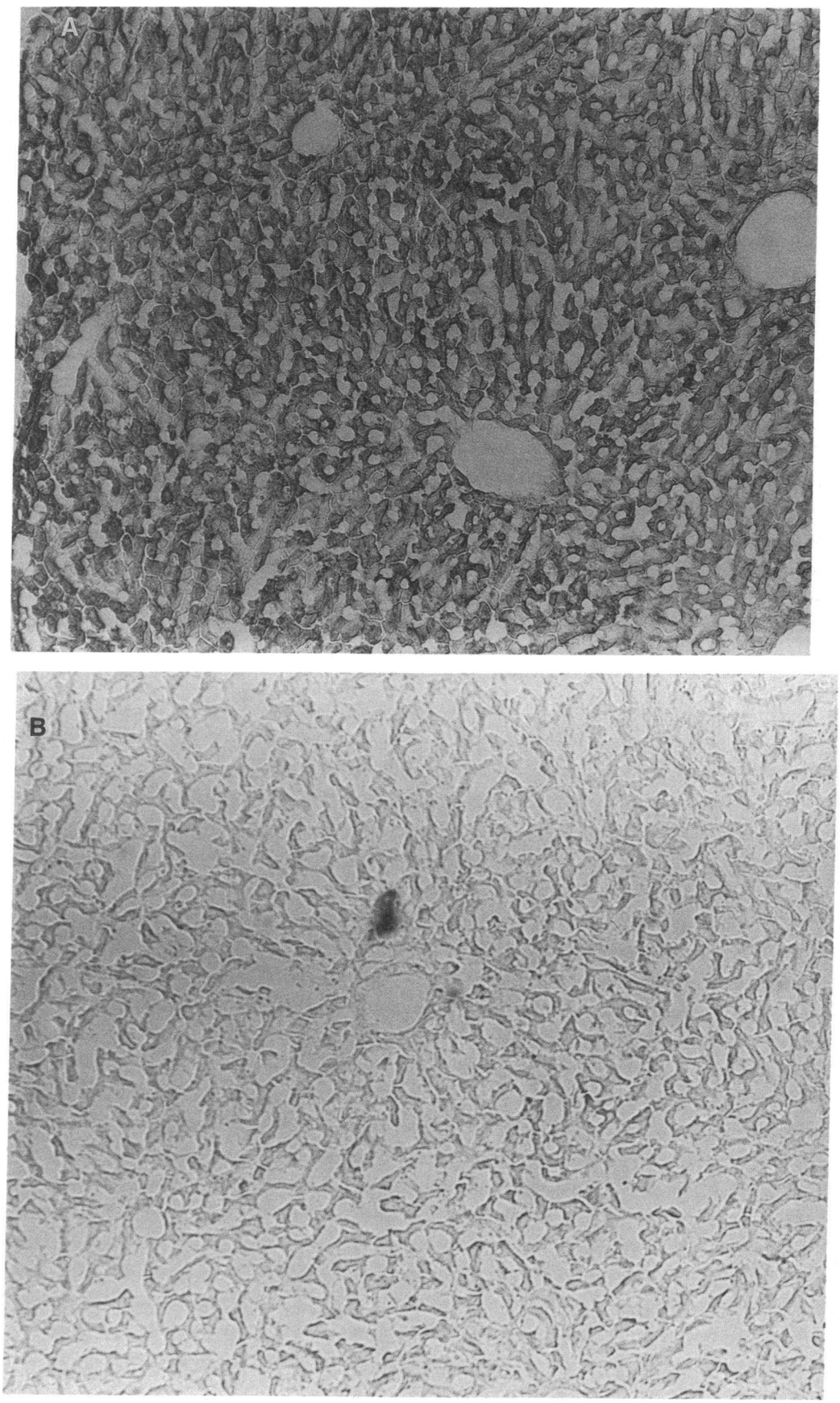

Figure 2. Immunohistochemical detection of albumin in liver sections of CHO and TNFα mice. 34 d postinoculation, frozen liver sections of CHO mice (*A*) and TNFα mice (*B*) were incubated with affinity purified antibodies to murine albumin, as described in Methods. ×100 (*A* and *B*).

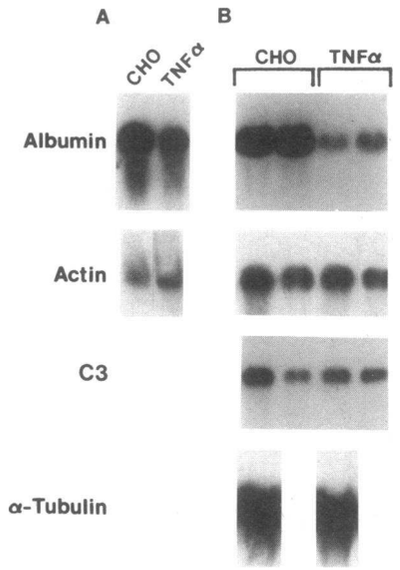

Figure 3. Northern blot analysis of albumin mRNA levels of livers from CHO and TNFα mice. 2 μg of poly A+ liver RNA from TNFα mice after the onset of weight loss (34 d, *A*) before the onset of weight loss (16 d, *B*), and from the respective CHO-control mice (*A* and *B*) were electrophoresed, transferred, and hybridized to radiolabeled cDNAs for murine albumin, β-actin, complement C3, or α-tubulin.

albumin gene transcription occurs before the onset of weight loss in this animal model.

Hypoalbuminemia has been examined in two other experimental conditions: acute fasting and acute inflammation. In fasted rats the decreased albumin synthesis results from a decrease in albumin mRNA and the amount of albumin mRNA bound to polyribosomes (25, 26). Upon refeeding, the albumin mRNA reverts to its normal distribution (28). A similar decrease in albumin synthesis and disaggregation of polyribosomes is found in perfused livers from fasted animals, both of which were reversed by the addition of ornithine or spermine to the perfusate (29). Although the disaggregation of liver polyribosomes has not been assessed in cachexia, the marked inhibition of albumin gene transcription appears to be the predominant mechanism for the decreased albumin synthesis demonstrated in this experimental model.

Acute inflammation induced by inoculation with either turpentine (2, 30) or bacterial lipopolysaccharide (31) causes a stimulation in the synthesis of acute phase reactants with a concomitant decrease in albumin mRNA levels. This decrease

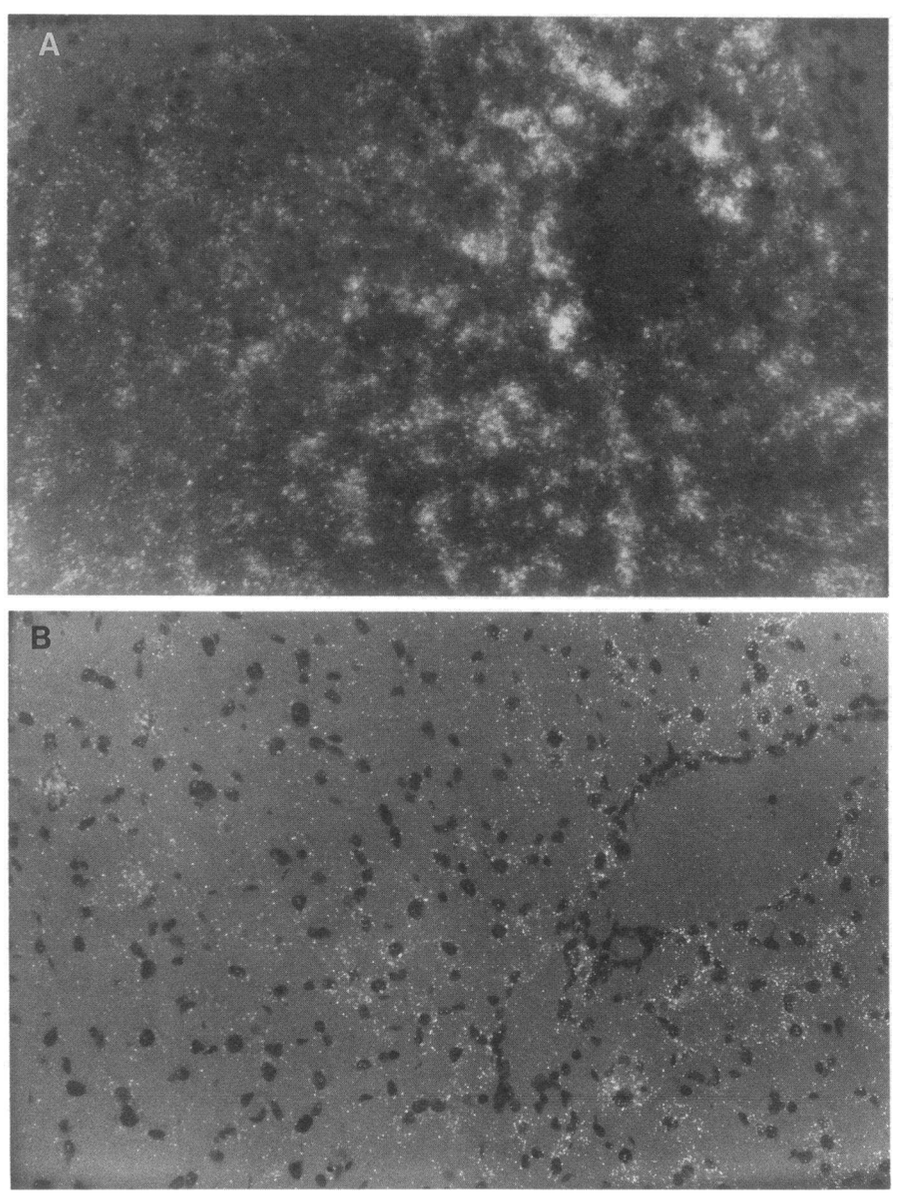

Figure 4. Distribution of albumin mRNA detected by in situ hybridization in the liver sections of CHO and TNFα mice. Before the onset of weight loss (16 d postinoculation) liver sections of CHO mice (*A* and *C*) and TNFα mice (*B* and *D*) were hybridized with ^{35}S-labeled albumin antisense RNA (*A* and *B*) or with ^{35}S-labeled albumin sense RNA (*C* and *D*). ×200.

in steady state mRNA levels is reproduced in vivo by the inoculation of monocytic products (2). In agreement with the in vivo finding, the monokine IL-1 decreases albumin synthesis and mRNA levels in primary murine hepatocyte cultures (28, 32). TNFα might have a direct role in albumin gene expression, since TNFα decreased albumin mRNA and albumin synthesis in human hepatoma cell lines (33, 34). However, in primary rat hepatocyte cultures, TNFα decreased albumin synthesis only by 10–30% (35). Thus, the effects of TNFα on albumin synthesis by normal hepatocytes in vitro are inconclusive. To our knowledge, no evaluation of the effects of TNFα on albumin gene expression has been performed previously.

Besides decreasing albumin synthesis in vivo, the acute phase response increases the synthesis of species-specific acute phase reactants, which include serum amyloid A, gamma fibrinogen, several complement proteins and α2 macroglobulin in the mouse (31, 32). Although TNFα is capable of inducing a limited subset of acute phase reactants in vitro (32–35), the serum levels of these proteins are not elevated in cachexia-associated chronic diseases. In the TNFα-secreting cachectic mice, the hepatic steady state mRNA levels of α2 macroglobulin, gamma-fibrinogen, and C3 are not increased. Therefore, the TNFα mice represent a good model for the cachexia of chronic disease.

We (23) and others (24, 25) have previously demonstrated that weight loss induced by fasting is associated with decreased albumin synthesis and mRNA levels in rats. Therefore, the decreased albumin synthesis and mRNA levels in the cachectic TNFα mice could be related to either weight loss or other factors including TNFα. To eliminate the confounding effect of weight loss on albumin gene expression, we studied TNFα mice before the onset of weight loss. TNFα markedly inhibited albumin gene transcription despite the absence of weight loss. These findings indicate that TNFα may be responsible, at least in part, for the inhibition of albumin synthesis that is characteristic of cachexia-associated infection, inflammation, and cancer, all of which manifest elevated TNFα levels. To the extent that our experimental conditions reflect human cachexia, our results provide an alternative hypothesis to the

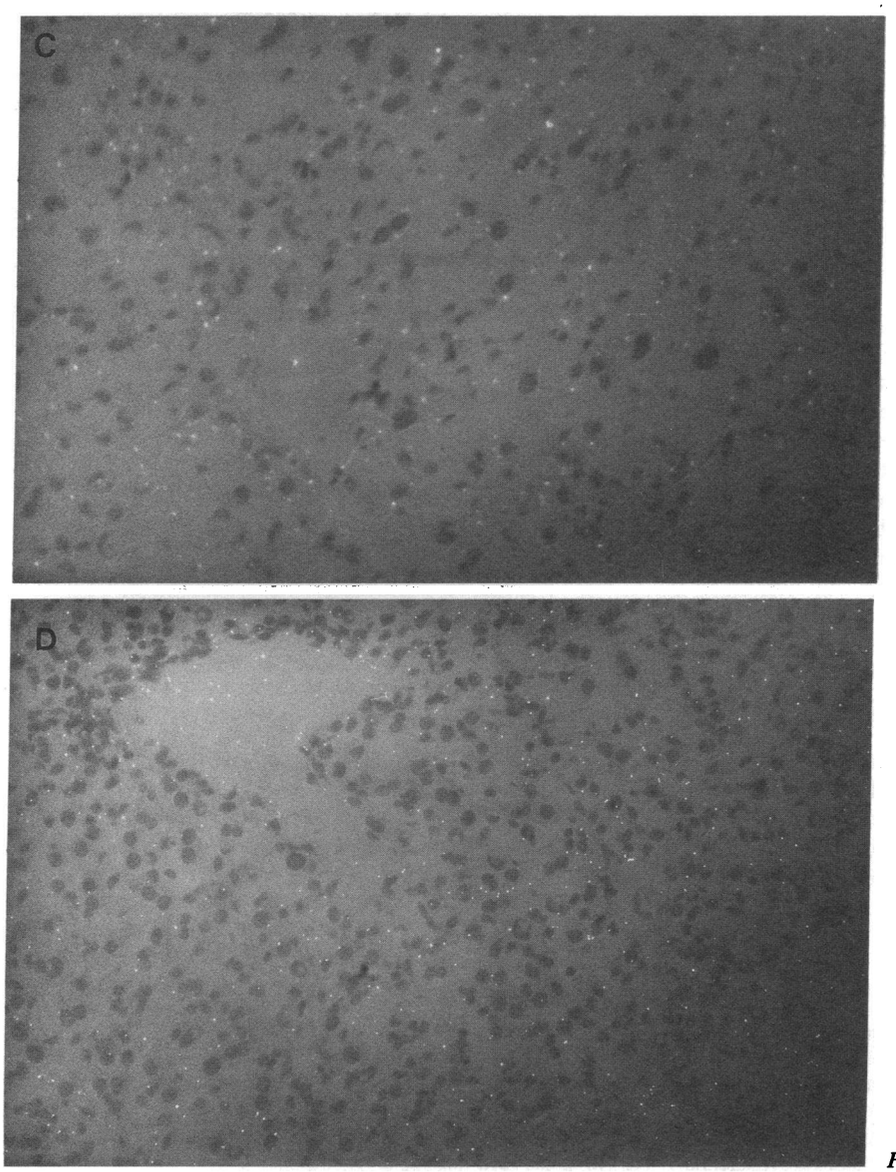

Figure 4 (Continued)

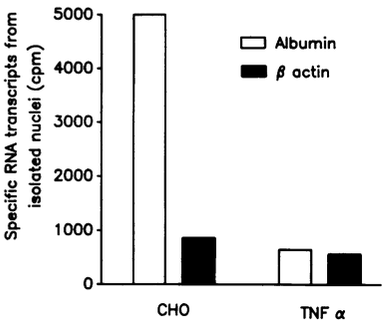

Figure 5. Run-off transcriptional assay from nuclei isolated from the livers of CHO and TNFα mice at 16 d postinoculation before the onset of weight loss. Purified radiolabeled nascent RNA (2×10^7 cpm) from hepatic nuclei were hybridized to 0.5 μg of each cDNA insert immobilized on nitrocellulose filters. The amount of hybridization was quantitated by liquid scintillation spectoscopy for individual cDNAs.

concept that malnutrition and weight loss are the major factors responsible for the depressed albumin synthesis in cachectic patients.

In vivo, TNFα might be acting directly by binding to its hepatocyte receptor or indirectly by stimulating the release of a second agonist, such as IL-1 or IL-6 (37, 38). IL-1 is also able to inhibit albumin synthesis in vitro (35). However, neither IL-1 nor IL-6 levels have been evaluated in cachexia. The interactions of these polypeptides can be specifically evaluated in primary hepatocyte cultures.

If TNFα directly inhibits albumin gene expression, then future studies must identify the intracellular messenger that mediates this response. We have previously shown that TNFα can modulate transcription through the AP-1 binding site by activating protein kinase C and stimulating the synthesis of the transcriptional factors JUN/AP-1 and FOS (39). In other systems, TNFα stimulates transcription in a protein kinase C independent pathway that might involve cyclic AMP (38). Recent studies have demonstrated that TNFα can also stimulate transcription by increasing nuclear factor binding to the NF-KB site (40). Further studies may reveal that additional intracellular pathways are activated by TNFα.

A series of positive and negative regulatory regions in the albumin gene have already been characterized by gene transfer, cell-free transcription, and protein binding assays (21, 41–43). Several DNA-binding proteins interact with the albumin promoter and are required for liver-specific control of albumin gene transcription. TNFα may modify these hepatic DNA-binding proteins, thereby inhibiting albumin gene transcription. This animal model of cachexia provides a unique opportunity to assess whether these transcriptional factors are affected in vivo by TNFα.

TNFα inhibits albumin gene expression independent of weight loss in this experimental model, and TNFα levels are elevated in patients with cachexia-associated diseases. Therefore, some of the metabolic changes including hypoalbuminemia that characterize cachectic patients might reflect the effects of TNFα.

Acknowledgments

We thank Linda Veloz, Michael Filip, and Charles Johnson for their excellent technical assistance, Andrea Arata for the typing of this manuscript, and Braun Brelin for preparing the graphics.

This work was supported by United States Public Health Service Grants DK07202, DK38652, and GM41804, grants from the Veterans Administration, and a University of California at San Diego Academic Senate research award. D. A. Brenner is a Pew Scholar in the Biomedical Sciences, M. Chojkier is a recipient of a Research Career Development Award, Veterans Administration, and M. Buck and S. Feitelberg were American Liver Foundation Fellows.

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