REFERENCES

1. Alter HJ. Descartes before the horse: I clone, therefore I am: the hepatitis C virus in current perspective. Ann Intern Med 1991; 115:644-649.
2. Esteban JI, Lopez-Talavera JC, Genesca J, Madoz P, Viladomil L, Muniz E, Martin-Vega C, et al. High rate of infectivity and liver disease in blood donors with antibodies to hepatitis C virus. Ann Intern Med 1991;115:443-449.
3. Alter MJ, Coleman PJ, Alexander WJ, Kramer E, Miller JK, Mandel E, Hadler SC, et al. Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. JAMA 1989;262:1201-1205.
4. Gumbo ME, Alter HJ, Aledort LM, Quan S, Hatzakis A, Goedert JJ. Heterosexual cotransmission of hepatitis C virus and human immunodeficiency virus. Ann Intern Med 1991;115:764-768.
5. Wejstal R, Hermodsson S, Iwarson S, Norkrans G. Mother to infant transmission of hepatitis C virus infection. J Med Virol 1990;30:178-180.
6. Thaler MM, Park CK, Landers DV, Wara DW, Houghton M, Veereman-Wauters G, Sweet RL, et al. Vertical transmission of hepatitis C virus. Lancet 1991;338:17-18.
7. Melbye M, Biggar RJ, Wantzin P, Krogsgaard K, Ebbesen P, Becker NG. Sexual transmission of hepatitis C virus: cohort study (1981-9) among European homosexual men. BMJ 1990;301: 210-212.
8. Szumness W, Steven CE, Harley EJ, Zang EA, Oleszkowr R, Williams DC, Sadovsky R, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled trial in a high risk population in the United States. N Engl J Med 1980;303:833-841.
9. Kolho E, Naukkarinen R, Ebeling F, Rasi V, Ikkala E, Krusius. Transmission of hepatitis C virus to sexual partners of seropositive patients with bleeding disorders: a rare event. Scand J Infect Dis 1991;23:667-670.
10. Shev S, Wejstal R, Wahl M, Hermodsson S, Norkrans G. The lack of transmission of NANB/C hepatitis between acute and chronically infected patients and their heterosexual partners. Scand J Infect Dis 1991;23:407-411.
11. Everhart J, DiBisceglie AM, Murray LM, Alter HJ, Melpolder JJ, Kou G, Hoofnagle JH. Risk of nonA nonB (type C) hepatitis through sexual or household contact with chronic carriers. Ann Intern Med 1990;112:544-545.
12. van Doornum GJJ, Hooykaas C, Cuypers MT, van der Linden MMD, Coutinho RA. Prevalence of hepatitis C virus infections among heterosexuals with multiple partners. J Med Virol 1991: 35:22-27.
13. Hsu HH, Wright TL, Luba D, Martin M, Feinstone SM, Garcia G, Greenberg HB. Failure to detect hepatitis C virus genome in human secretions with the polymerase chain reaction. HEPATOLOGY 1991;14:763-767.
14. Wang JT, Wang TH, Sheu JC, Lin JT, Chen DS. Hepatitis C virus RNA in saliva of patients with posttransfusion hepatitis and low efficiency of transmission among spouses. J Med Virol 1992;36: 28-31.

DOES ALBUMIN REGULATE ALBUMIN?

Pietrangelo A, Panduro A, Roy Chowdhury JR, Shafritz DA. Albumin gene expression is down-regulated by albumin or macromolecule infusion in the rat. J Clin Invest 1992;89:1755-1760.

ABSTRACT

A novel feedback regulatory mechanism operating on transcription of the albumin gene is described in the rat. In 1946, it was proposed that circulating colloids, including serum albumin, may affect the synthesis and/or secretion of albumin in the liver. The molecular basis for this proposed regulation has now been investigated by adding oncotically active macromolecules to the circulation of normal or genetically albumin-deficient Nagase analbuminemic rats (NAR) and analyzing the hepatic expression of genes, including albumin after 24 h. The transcription rate of the albumin gene was higher in NAR than in normal rats and was dramatically reduced by raising serum albumin to 1.6 g/dl. Intravenous infusion of albumin into normal rats also decreased transcriptional activity of the albumin gene by 50-60%, and this decrease correlated with changes in serum colloid osmotic pressure after albumin infusion. Inhibition of albumin gene transcription was also observed upon intravenous infusion of other protein or nonprotein macromolecules, such as γ-globulin and dextran. This down-regulation appears to control the steady-state level of albumin mRNA in the liver. Aside from a concomitant decrease in apo E gene transcription after albumin or macromolecule infusion, there was no change in the transcription rate of other genes, including those exhibiting liver-preferred or -specific expression (e.g., tyrosine aminotransferase, cytochrome P-450, α_1-antitrypsin, apolipoproteins A-I and B, and transferrin) or general cellular expression (e.g., α-tubulin, pro α_2 collagen, and β-actin). Feedback regulation of albumin gene expression by serum colloids may serve as a specific homeostatic mechanism to maintain the steady-state level of total protein in the circulation.

COMMENTS

Several decades ago, it was noted that an infusion of dextran or an elevation in γ-globulins, such as that which developed after an immunization, resulted in hypoalbuminemia (1, 2). These clinical observations formed the basis for the proposal that colloid osmotic pressure, rather than serum albumin levels, regulated the synthetic rate of albumin (3). This proposal was then tested with a series of experiments performed in rabbits and isolated perfused rat livers. Dextran infusions (3) or hypergammaglobulinemia (4) in rabbits caused a decrease in the plasma albumin concentration with a shift, in the dextran model, of intravascular albumin into the extravascular compartment. In addition, the total albumin pool (i.e., the albumin present in both the intravascular and extravascular compartments) decreased despite a lower rate of degradation of albumin. The decrease in pool size was thus proposed to be secondary to a decrease in albumin synthesis (3, 4). Therefore in these experiments the data suggested that the regulation of the rate of albumin synthesis was independent of the plasma albumin concentration and the total albumin pool size. Instead, the rate of albumin synthesis may have been regulated by a mechanism common to both experimental manipulations. Because both dextran and γ-globulin are similar in that they are macromolecules capable of altering colloid osmotic pressure, this supported the proposal that albumin synthesis was regulated by colloid osmotic pressure. Changing the colloid osmotic pressure in the intact animal could potentially cause a series of events (intrahepatic, extrahepatic or both) that would result in the alteration of the albumin synthetic rate. The isolated

perfused rat liver model was tested to determine whether the liver was directly responsive to the changes in colloid osmotic pressure or whether extrahepatic signals were involved. The results obtained with this model were similar to those obtained in intact animals (5). When livers were perfused with blood that was low in protein (after plasmapheresis), the rate of albumin synthesis doubled. In contrast, when the colloid osmotic pressure of the low-protein blood was restored to a level equivalent to that of plasma using dextran, albumin synthesis was similar to that of controls. In this model, the albumin synthetic rate was manipulated despite consistently low blood albumin levels by altering the content of colloid in the blood, thus providing further support for the proposal that colloid osmotic pressure regulated the synthetic rate of albumin.

The term "albumin synthesis" used thus far includes all steps necessary for the production and secretion of albumin into either the blood or the extravascular compartment. The molecular mechanisms (i.e., transcription, posttranscriptional processing, translation, storage or secretion) responsible for the alterations in the rate of albumin synthesis that may be secondary to changes in colloid osmotic pressure are unknown. Pietrangelo and colleagues used an *in vivo* molecular approach in the rat to test the hypothesis that colloid infusions altered the rate of albumin synthesis and further define the molecular mechanism(s) responsible for the observed alteration in synthetic rate. Three different macromolecules were administered to either Sprague-Dawley rats (SDR) or Nagase analbuminemic rats (NAR). The NAR are a line of rats established from SDR in which the analbuminemia is inherited as an autosomal recessive trait (6). NAR have similar levels of total protein and slightly lower levels of colloid osmotic pressure compared with SDR, but NAR albumin levels are approximately 1/1000 that of SDR. Initially, serum colloid osmotic pressure was measured in both types of rats at baseline and at 2 and 24 hr after an albumin infusion. Colloid osmotic pressure increased to a similar degree in both types of rats after the albumin infusion. The basal transcriptional activity of the albumin gene was then evaluated and was found to be twice as high in NAR as compared with SDR. Twenty-four hours after the infusion of either albumin, dextran or γ-globulin, the relative transcription rate of several genes expressed in the liver was determined. The genes tested included the liver-specific genes (albumin, fibrinogen, tyrosine aminotransferase and α_1-antitrypsin), the liver-enriched genes (apoproteins B and AI and transferrin), the broadly expressed genes (β-actin, α-tubulin and α_2-collagen) and the acute-phase reactant genes (fibrinogen and α_1-acid glycoprotein). Control animals were infused with crystalloid (normal saline) rather than colloid. The albumin infusions inhibited the relative rate of transcription of the albumin gene by 50% to 60% in both the SDR and the NAR as assessed by densitometric analysis. The relative transcription rates of the other genes were not altered. The results obtained after the dextran and γ-globulin infusions were similar to those obtained with albumin. The levels of albumin messenger RNA decreased after albumin and dextran infusion, paralleling that of transcription. This study nicely demonstrated that the changes in the rate of albumin synthesis in response to macromolecule infusions was secondary, at least in part, to a decrease in the relative rate of transcription of the albumin gene. The change in transcriptional activity was also reflected in the quantity of albumin messenger RNA detected, as assessed by Northern-blot analysis. Further studies will be necessary to explain whether the colloid osmotic pressure directly regulates the changes in albumin synthesis. It will also be of interest to determine the regulatory factors or signals responsible for the changes observed in the relative rate of transcription of the albumin gene.

In addition to providing new insight into the molecular mechanisms that are responsible for the physiological regulation of albumin gene expression *in vivo*, the article also serves as an important reminder that serum albumin levels do not reflect hepatic synthetic function. In the studies presented above, in both the rabbit and the rat, the concentration of serum albumin was manipulated by infusing macromolecules, by altering the colloid osmotic pressure or both. A potentially similar example in human beings may be observed in patients who have hypoalbuminemia and hypergammaglobulinemia secondary to chronic liver disease. Clinically, the low serum albumin has been interpreted as representing altered or damaged hepatic albumin production. Several studies have revealed that this interpretation may not be correct. When the intravascular albumin pool was evaluated in patients with cirrhosis and hypoalbuminemia, the results obtained were variable and many patients fell within the normal range (7). In those patients with cirrhosis, hypoalbuminemia and ascites, measurements of the total albumin pool were all in the normal range (7). Furthermore, the serum proteins and albumin were depressed, and the globulins were elevated to the same levels whether the patients studied synthesized albumin at a high or a low rate (8). The synthetic rate of albumin did not correlate with the other conventional "liver function tests" or with standard clinical criteria used to assess hepatic function (9). Therefore a basic problem when evaluating hepatic function in patients is that a generally accepted reliable reference value is still lacking. The blood liver chemistries and clinical criteria used to assess such patients are insufficient to predict the course or the prognosis in each individual patient. Serum albumin concentration represents the net result of synthesis, degradation and distribution (intravascular and extravascular). It is neither a good index of hepatic albumin synthesizing capacity nor the overall synthetic capacity of the liver in cirrhosis. Although the serum albumin level may be significantly depressed in patients with severe liver disease, the rate of albumin synthesis may be increased.

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REFERENCES

1. Carbone JV, Uzman LL, Plough IC. Changes in serum proteins produced by infusions of dextran. Proc Soc Exp Biol 1955;90: 68-70.
2. Bjorneboe M, Schwartz M. Investigations concerning the changes in serum proteins during immunization: the cause of hypoalbuminemia with high gamma globulin levels. J Exp Med 1959;110: 259-270.
3. Rothschild MA, Oratz M, Wimer E, Schreiber SS. Studies on albumin synthesis: the effects of dextran and cortisone on albumin metabolism in rabbits studied with albumin-I^{131}. J Clin Invest 1961;40:545-554.
4. Rothschild MA, Oratz M, Franklin EC, Schreiber SS. The effect of hypergammaglobulinemia on albumin metabolism in hyperimmunized rabbits studied with albumin-I^{131}. J Clin Invest 1962;41: 1564-1571.
5. Tracht ME, Tallal L, Tracht DG. Intrinsic hepatic control of plasma albumin concentration. Life Sci 1967;6:2621-2628.
6. Nagase S, Shimamune K, Shumiya S. Albumin-deficient rat mutant. Science 1979;205:590-591.
7. Wilkinson P, Mendenhall CL. Serum albumin turnover in normal subjects and patients with cirrhosis measured by ^{131}I-labelled human albumin. Clin Sci 1963;25:281-292.
8. Rothschild MA, Oratz M, Zimmon D, Schreiber S, Weiner I, Van Caneghem A. Albumin synthesis in cirrhotic subjects with ascites studied with carbonate-^{14}C. J Clin Invest 1969;48:344-350.
9. Hasch E, Jarnum S, Tygstrup N. Albumin synthesis rate as a measure of liver function in patients with cirrhosis. Acta Med Scand 1967;182:83-92.

ADH1, 2 AND *3*: GENES WHOSE TIMES HAVE COME

van Ooij C, Snyder RC, Paeper BW, Duester G. Temporal expression of the human alcohol dehydrogenase gene family during liver development correlates with differential promoter activation by hepatocyte nuclear factor 1, CCAAT/enhancer-binding protein α, liver activator protein, and D-element-binding protein. Mol Cell Biol 1992;12:3023-3031.

ABSTRACT

The human class I alcohol dehydrogenase (ADH) gene family consists of *ADH1*, *ADH2*, and *ADH3*, which are sequentially activated in early fetal, late fetal, and postnatal liver, respectively. Analysis of ADH promoters revealed differential activation by several factors previously shown to control liver transcription. In cotransfection assays, the *ADH1* promoter, but not the *ADH2* or *ADH3* promoter, was shown to respond to hepatocyte nuclear factor 1 (HNF-1), which has previously been shown to regulate transcription in early liver development. The *ADH2* promoter, but not the *ADH1* or *ADH3* promoter, was shown to respond to CCAAT/enhancer-binding protein α (C/EBPα), a transcription factor particularly active during late fetal liver and early postnatal liver development. The *ADH1*, *ADH2*, and *ADH3* promoters all responded to the liver transcription factors liver activator protein (LAP) and D-element-binding protein (DBP), which are most active in postnatal liver. For all three promoters, the activation by LAP or DBP was higher than that seen by HNF-1 or C/EBPα, and a significant synergism between C/EBPα and LAP was noticed for the *ADH2* and *ADH3* promoters when both factors were simultaneously cotransfected. A hierarchy of ADH promoter responsiveness to C/EBPα and LAP homo- and heterodimers is suggested. In all three ADH genes, LAP bound to the same four sites previously reported for C/EBPα (i.e., −160, −120, −40, and −20 bp), but DBP bound strongly only to the site located at −40 bp relative to the transcriptional start. Mutational analysis of *ADH2* indicated that the −40 bp element accounts for most of the promoter regulation by the bZIP factors analyzed. These studies suggest that HNF-1 and C/EBPα help establish ADH gene family transcription in fetal liver and that LAP and DBP help maintain high-level ADH gene family transcription in postnatal liver.

COMMENTS

A major problem in biological studies is to understand how genes are switched on and off during development and how certain genes remain active at high levels in adult tissues. Broadly speaking, these processes depend on the nucleotide sequence of gene promoters (*cis*-acting elements) and protein transcription factors (*trans*-acting factors). The liver has been an attractive model to study these questions because of its size, accessibility and the large number of well-characterized, abundant proteins (mainly enzymes and plasma proteins) that are made predominantly in the liver. Studies on the promoters for the albumin, α_1-antitrypsin, fibrinogen, C-reactive protein and now alcohol dehydrogenase genes have revealed a number of transcription factors involved in their expression (Table 1).

CCAAT/enhancer binding protein (C/EBP) is named for its binding to the CCAAT sequence motif and certain viral enhancer sequences, and several numbers of this family have been identified. These include C/EBPα (1), liver activator protein (LAP or C/EBPβ) (2), C/EBPγ (or Ig/EBP-1) (3), C/EBPδ (4), CRP1 and CRP3 (C/EBP-related proteins 1 and 3) (5). These factors have been designated bZIP proteins because of their *b*asic DNA binding region and their "leucine *zip*per" (a region in which leucine occurs as every seventh residue), which allows the protein to homodimerize and to heterodimerize with other members of this family of proteins (4, 5). Outside of these two domains, the factors are not very homologous. A related protein, D-binding protein (DBP), is named for its ability to bind to the D region of the albumin promoter (6). It is a bZIP protein with a degenerate leucine zipper that will not heterodimerize with other leucine zipper proteins, such as C/EBPα or β. Interestingly, an inhibitory factor, liver inhibitory protein (LIP), is translated from the same messenger RNA (mRNA) as LAP, using a downstream AUG as the initiator codon. LIP binds DNA at the same site as LAP (perhaps with higher affinity) but does not activate transcription. The ratio of LAP/LIP may play a role in controlling transcription of certain genes, and this ratio rises sharply immediately prenatally (7). The bZIP factors are encoded by genes having no or few introns and are also expressed at lower levels in adipose tissue and lung. Of interest, the C/EBPβ proteins were first identified as participants in the response of acute-phase reactant genes to interleukin-1 and interleukin-6 (8).

Another major liver-specific transcription factor is hepatocyte nuclear factor-1α (HNF-1α). This protein