HETEROGENEOUS LOBULAR DISTRIBUTION OF HEPATOCYTES EXPRESSING ACUTE-PHASE GENES DURING THE ACUTE INFLAMMATORY REACTION

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Although a zonal distribution of hepatocytes expressing various metabolic functions in liver lobule has been described (1), it is still debated whether functional heterogeneity in the lobule also occurs with respect to plasma protein synthesis, an important function of hepatocytes. Acute-phase reactants are a group of secretory proteins with dramatically altered rates of synthesis during the acute-phase response of the liver (2). In this article we analyze the hepatic cellular distribution of the mRNA and protein products of three acute-phase genes, α_2-macroglobulin (α_2M), α_1-inhibitor 3 (α_1I3), and α_1-antitrypsin (α_1PI). α_2M is a major acute-phase reactant in rats with drastically increased hepatic mRNA concentrations after turpentine injection (3). Concomitantly, the steady-state levels of α_1I3 mRNA are decreased (4). α_1PI is only a moderately reacting acute-phase protein in the rat (5). Here we investigate specifically whether hepatocytes expressing these three genes show a specialized location in normal rat livers and whether their topographic distribution is altered during the acute inflammatory reaction (AIR).

Materials and Methods

Animals. Adult male Sprague-Dawley rats (180–230 g) (Charles River Breeding Laboratories, St. Aubin-les-Elbeuf, France) were used. An AIR was induced by a subcutaneous injection of turpentine (0.5 ml per 100 g body weight) (Prolabo, Paris, France). The animals were killed at 0, 4, 8, 12, 18, 24, 48, and 72 h after induction of the AIR. Three rats per time point were used for in situ hybridization (ISH) and immunoperoxidase experiments.

Molecular Probes. The α_2M (pRLA2M/29J) cDNA probe and the α_1I3 (pRLA12/23J) cDNA probe were prepared as described (3, 4). The α_1PI (pliv 3) cDNA probe (6) was provided by Dr. M. Weiss (Institut Pasteur, Paris, France).

In Situ Hybridization. Liver fixation and preparation of paraffin sections were made as previously detailed (7). The sections were fixed in 4% paraformaldehyde and hybridized as previously reported (7). The probes were labeled by nick translation to a specific activity of 1–2 × 10^8 cpm/μg DNA. Each section was hybridized with 10^5–10^6 cpm of the radioactive

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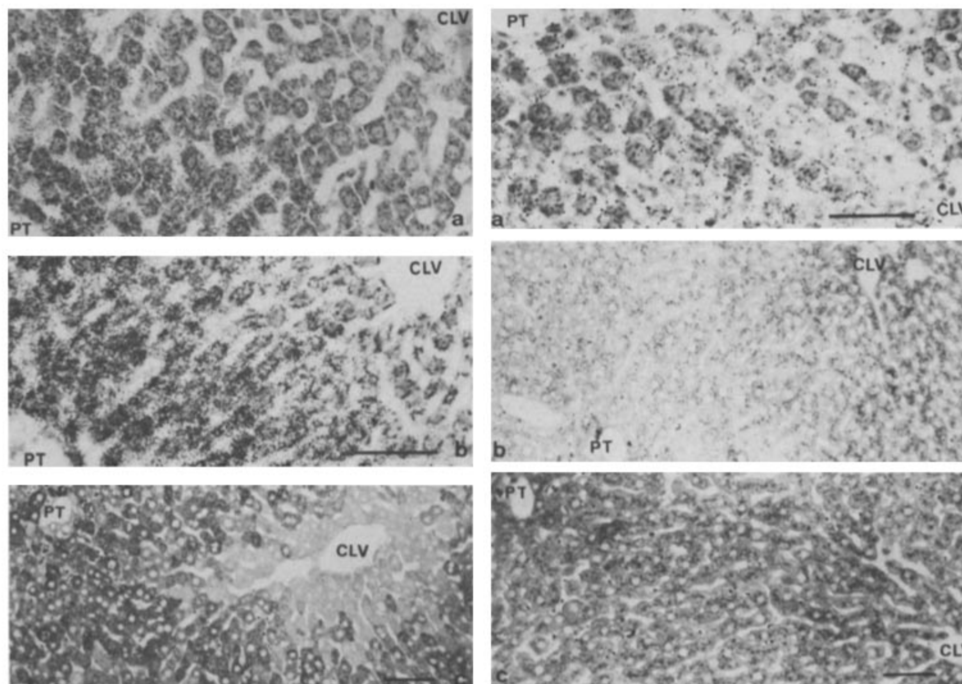
probe at 42°C for 24 h. A quantitative analysis was made by optical grain counts, according to the morphometric method previously described (7). For each time point, at least two different slides from two different animals were evaluated. RNase controls were performed by incubation with RNase A (Sigma Chemical Co., St. Louis, MO) before hybridization with a specific probe. pBR controls were performed by hybridizing tissue sections with a ^{35}S-labeled pBR 325 fragment.

Immunoperoxidase Staining. Liver sections adjacent to those used for ISH were processed for detection of α_2M, α_1I3, and α_1PI with an indirect immunoperoxidase method using specific antisera (5, 8, 9). For control reactions, sections were incubated with peroxidase-labeled anti-rabbit Ig or directly placed in the diamino-benzidine medium.

Results

Analysis by ISH. In normal liver α_2M, α_1I3, and α_1PI mRNA were detected in all hepatocytes. The signal for α_1I3 mRNA was high, while α_2M (Fig. 1 *a*) and α_1PI mRNA appeared less abundant. Furthermore, while the signal for α_1PI mRNA was homogeneously distributed within the liver lobule, the distribution of the signal for



FIGURES 1 and 2. (*Fig. 1, left*) Localization of α_2M gene products. (*a*) Localization of mRNA in normal liver. Silver grains are more numerous in PP and ML hepatocytes than in PV hepatocytes. (*b*) Localization of α_2M mRNA 18 h after AIR induction. The hybridization signal is dramatically increased over PP and ML hepatocytes. (*c*) Immunoperoxidase localization of α_2M 18 h after AIR induction. A strong staining is visible in PP and ML hepatocytes, while PV hepatocytes are negative. PT, portal tract; CLV, centrilobular vein. Bars: (*a* and *b*) 50 μm, (*c*) 100 μm. (*Fig. 2, right*) Localization of α_1PI gene products. (*a*) Localization of mRNA in normal liver. All hepatocytes are labeled with an homogeneous distribution in the lobule. (*b*) Immunoperoxidase localization of α_1PI in normal liver. All hepatocytes in the lobule are reactive, with a more intense staining in PV hepatocytes. (*c*) Immunoperoxidase localization of α_1PI 24 h after AIR induction. The staining intensity is increased, and is now homogeneously distributed across the lobule. PT: portal tract. CLV: centrilobular vein. Bars: (*a*) 50 μm, (*b* and *c*) 100 μm.

α_1I3 and α_2M was uneven over the three zones of the lobule. Silver grains corresponding to α_2M mRNA (Fig. 1 *a*) and α_1I3 mRNA were more numerous in the periportal (PP) and mediolobular (ML) area than in the perivenous (PV) areas. 4 h after induction of the AIR, several sparsely distributed PP hepatocytes displaying a dramatic increase in the signal level for α_2M mRNA were observed. At later times the number of hepatocytes with a high signal level per cell increased progressively. At the maximum of the AIR (18 h) nearly all hepatocytes of the PP and ML areas were intensely labeled, whereas the signal level in the PV areas remained mostly unchanged (Fig. 1 *b*). By contrast, as early as 4 h after induction of the AIR, α_1I3 signal levels were decreased in the PV and ML areas and were still low at 24 and 72 h. Finally, the signal for α_1PI mRNA increased moderately over all hepatocytes between 4 and 18 h after induction of the AIR, and the distribution of the signal within the lobule remained homogeneous at all times tested (Fig. 2 *a*).

Optical counts revealed an increase in the number of α_2M mRNA in PP hepatocytes during the AIR of about threefold and fourfold for the nuclear and cytoplasmic counts, respectively (Fig. 3 *a*). In PV areas the nuclear signal level remained unchanged at all times tested, with moderate fluctuations in the cytoplasmic counts during the AIR. With regard to α_1I3 expression, a decrease of nuclear and cytoplasmic counts was recorded in PP hepatocytes 4 h after induction of the AIR, reaching a 6-fold decrease of the nuclear counts and a 3.4-fold decrease of the cytoplasmic

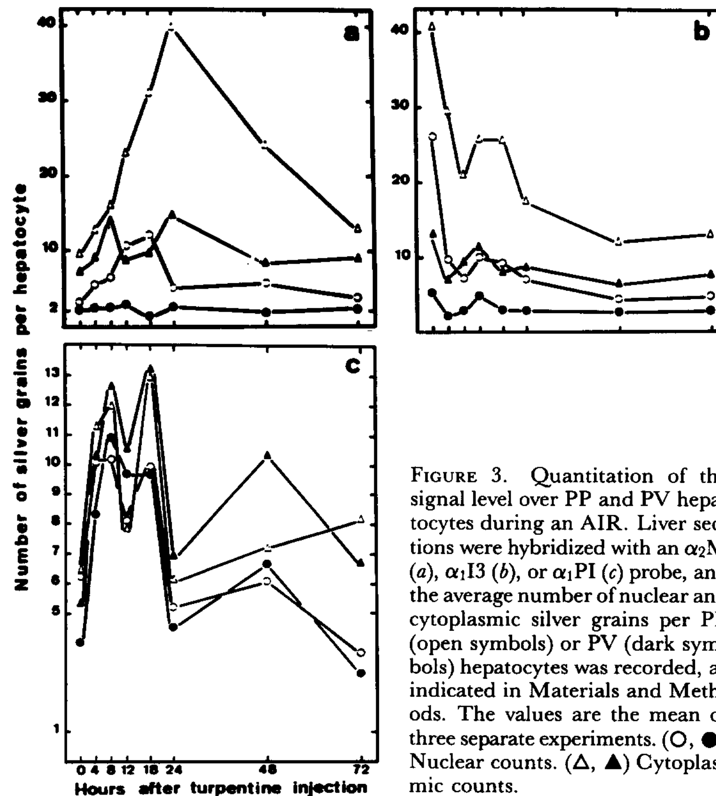


FIGURE 3. Quantitation of the signal level over PP and PV hepatocytes during an AIR. Liver sections were hybridized with an α_2M (*a*), α_1I3 (*b*), or α_1PI (*c*) probe, and the average number of nuclear and cytoplasmic silver grains per PP (open symbols) or PV (dark symbols) hepatocytes was recorded, as indicated in Materials and Methods. The values are the mean of three separate experiments. (○, ●) Nuclear counts. (△, ▲) Cytoplasmic counts.

counts by 48 h (Fig. 3 *b*). Only slight fluctuations of the nuclear and cytoplasmic counts were visible over PV hepatocytes. For α_1PI, the increase of the signal was small (~2-fold for the nuclear and cytoplasmic counts) and of the same magnitude in the two lobular zones (Fig. 3 *c*).

Immunoperoxidase Staining. In normal liver a weak α_2M immunostaining was detected exclusively in PP and ML hepatocytes. All hepatocytes stained for α_1I3 in the normal liver, except for a narrow ring of PV hepatocytes. All hepatocytes stained for α_1PI, with a more intense staining in PV hepatocytes (Fig. 2 *b*). During the AIR, the staining intensity for α_2M increased progressively, and by 18–24 h almost all hepatocytes in the PP and ML zones were strongly stained (Fig. 1 *c*). Concomitantly, the intensity of staining for α_1I3 decreased progressively in PP and ML areas. PV hepatocytes remained negative for both proteins during the AIR. The lobular distribution of α_1PI^+ hepatocytes changed towards a homogeneous pattern between 12 and 48 h after induction of the AIR (Fig. 2 *c*).

Discussion

Our study demonstrate that the location of a hepatocyte within the lobule influences the level at which it expresses the three acute-phase genes studied via pre- and/or posttranslational mechanisms, depending on the gene.

In normal liver, we observed that PP and ML hepatocytes contained much more α_1I3 and α_2M mRNA and protein than PV hepatocytes, although a low level of expression of the two genes was also detected in these cells by ISH. These observations suggest that as yet unknown factors, linked to the location of heptocytes within PP and ML microenvironments, influence the basal transcription rates and/or mRNA stabilities of the α_2M and α_1I3 genes. During the AIR, the α_2M gene was stimulated early in PP hepatocytes with a later recruitment of ML hepatocytes, suggesting that the signals for α_2M induction spread from the PP to the PV zones. These observations, in agreement with earlier reports on the morphologic kinetics of α_2M and C-reactive protein syntheses in the liver (10, 11), are consistent with the interpretation that the AIR mediators spread from the portal vein through the entire lobule. Similarly the decrease in α_1I3 mRNA during the AIR was shown to take place in the PP and ML zones. The pattern of lobular expression of the α_1PI gene in normal and stimulated livers differed significantly from that of the α_2M and α_1I3 genes. Staining for the α_1PI protein was more intense in PV hepatocytes, as previously reported (5), whereas, the repartition of α_1PI mRNA was homogeneous across the lobule. This observation is compatible with the interpretation that some mechanism(s) affecting translational or posttranslational processing, stability, and/or secretion of α_1PI specifically operate in PV hepatocytes, leading to a more important accumulation of the α_1PI protein within these cells. We found no difference in the extent of α_1PI mRNA induction between PP and PV hepatocytes on the one hand, and between nuclear and cytoplamsic α_1PI RNA products on the other. The present data, together with our previous observations of lobular heterogeneity in the level of expression of the albumin and α-fetoprotein genes (12), suggest that functional lobular heterogeneity is a concept applicable not only to enzymatic or metabolic functions (1), but to plasma protein synthesis as well.

The molecular mechanisms responsible for differences in the expression of plasma protein genes in different areas of a liver lobule are presently unknown. While it

is clear that hormones released by macrophages participate in the regulation of acute-phase genes (2), it is not yet clear whether hormones are also responsible for the uneven expression of these genes across the liver lobule in unstimulated livers. Hepatocytes of the different lobular areas differ in at least two aspects: (*a*) They are surrounded by different microenvironments as a result of the unidirectional blood flow, which leads to decreasing concentrations of soluble mediators from the portal triads towards the centrilobular vein (13). (*b*) The concentrations of hormone receptors on the surface of hepatocytes may differ in hepatocytes located in the different lobular areas. In the future, it will be crucial to establish the in vivo distribution of receptors or other cellular components involved in the control of gene expression in hepatocytes across a liver lobule.

Summary

Functional heterogeneity in the lobule with regard to plasma protein synthesis is still debated. Therefore, we have localized in liver sections from normal rats and from rats with turpentine-induced AIR the mRNA and protein products of three genes with different alterations in their hepatic expression during an AIR: α_2M and α_1PI, two positively reacting acute-phase genes, and α_1I3, a negative acute-phase reactant. In normal liver, all hepatocytes expressed α_2M and α_1I3 mRNA, but a preferential expression of α_2M and α_1I3 mRNA and protein in the PP and ML zones was observed. During an AIR, the level of α_2M mRNA increased fourfold in the cytoplasm of PP and ML hepatocytes, while the level of cytoplasmic α_1I3 mRNA was decreased about fourfold in the same zones, with parallel variations in the expression of the corresponding proteins. In contrast, no significant modulation of the RNA and protein concentrations of both genes was detected in PV areas. α_1PI mRNA was expressed at the same levels in the three lobular zones in normal liver, but staining for the α_1PI protein was more intense in the PV zones. During the acute-phase response α_1PI mRNA levels were increased twofold in all three lobular zones, and α_1PI staining became homogeneous within the lobule. These results demonstrate that the location of a hepatocyte with the liver lobule can influence the expression of the three genes under study both at pre- and post-translational levels, in basal conditions, as well as during modulation of their expression during the inflammatory reaction.

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References

1. Jungermann, K. 1986. Functional heterogeneity of periportal and perivenous hepatocytes. *Enzyme.* 35:161.
2. Fey, G., and J. Gauldie. 1989. The acute phase response of the liver in inflammation. *In* Progress in Liver Diseases. 9th edition. H. Popper and F. Schaffner, editors. Grune and Stratton, New York. In press.
3. Gehring, M. R., B. R. Shiels, W. Northemann, M. H. L. de Bruijn, C.-C. Kan, A. C. Chain, D. J. Noonan, and G. H. Fey. 1987. Sequence of rat liver α_2-macroglobulin and

acute phase control of its messenger RNA. *J. Biol. Chem.* 262:446.
4. Braciak, T. A., W. Northemann, G. O. Hudson, B. R. Shiels, M. R. Gehring, and G. H. Fey. 1988. Sequence and acute phase regulation of rat α_1-Inhibitor III messenger RNA. *J. Biol. Chem.* 263:3999.
5. Lamri, Y., S. Eriksson, A. Moreau, F. Gauthier, and G. Feldmann. 1986. Cellular and subcellular distribution of alpha-1-antitrypsin in the normal rat liver and during acute inflammatory reaction. A light and electron microscopic study with peroxidase labelled antibodies. *Cell. Mol. Biol.* 32:691.
6. Derman, E., K. Krauter, L. Walling, C. Weinberger, M. Ray, and J. E. Darnell, Jr. 1981. Transcriptional control in the production of liver specific-mRNAs. *Cell.* 23:731.
7. Bernuau, D., A. Poliard, and G. Feldmann. 1988. In situ cellular analysis of alpha-fetoprotein gene expression in regenerating rat liver after partial hepatectomy. *Hepatology.* 8:997.
8. Gauthier, F., and H. Mouray. 1976. Rat α_2-acute phase macroglobulin. Isolation and physicochemical properties. *Biochem. J.* 159:661.
9. Gauthier, F., and K. Ohlsson. 1978. Isolation and some properties of a new enzyme-binding protein in rat plasma. *Hoppe-Seyler's Z. Physiol. Chem.* 359:987.
10. Courtoy, P. J., C. Lombart, G. Feldmann, N. Moguilevsky, and E. Rogier. 1981. Synchronous increase of four acute phase proteins synthesized by the same hepatocytes during the inflammatory reaction. A combined biochemical and morphologic kinetics study in the rat. *Lab. Invest.* 44:105–115.
11. Kushner, I., and G. Feldmann. 1978. Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. *J. Exp. Med.* 148:466.
12. Poliard, A. M., D. Bernuau, I. Tournier, L. G. Legrès, D. Schoevaert, G. Feldmann, and J. M. Sala-Trepat. 1986. Cellular analysis by in situ hybridization and immunoperoxidase of alpha-fetoprotein and albumin gene expression in rat liver during the perinatal period. *J. Cell Biol.* 103:777.
13. Gumucio, J. J. 1989. Hepatocyte heterogeneity: the coming of age from the description of a biological curiosity to a partial understanding of its physiological meaning and regulation. *Hepatology.* 9:154.