Leading article – Hepatology series

Perisinusoidal lipocytes and fibrogenesis

Fibrosis of the liver results from a distortion of the rates of synthesis (fibrogenesis) and degradation (fibrolysis) of extracellular matrix molecules.\(^1\)\(^2\) It is now generally accepted that the disturbance of homeostasis of extracellular matrix metabolism in response to tissue injury is primarily caused by exaggerated matrix production but inhibition of the catabolic pathways of matrix components may provide additional mechanisms of extracellular matrix accumulation.\(^3\) The liver has a high regenerative capacity of parenchymal cells\(^4\) and normally only a small fraction (less than 0-6% of liver wet weight) of connective tissue, so a highly stimulated matrix production in response to injury seems to be an almost superfluous, functionally insufficient way of tissue repair.

Fibrosis comprises not only (a) a three to sixfold overall increase of extracellular matrix but also (b) a disproportionate increase in the five types of collagens identified so far in liver, of various proteoglycans (increase of dermatan/chondroitin sulphate to heparan sulphate ratio), and structural glycoproteins (fibronectin, tenasin, laminin, and other), (c) subtle changes of the microcomposition of certain extracellular matrix molecules (degree of hydroxylation of collagens and sulphation of glycosaminoglycans) and, clinically most significant, (d) the topographic redistribution of extracellular matrix, for example, in the space of Disse where it forms an incomplete basement membrane ('capillarisation' of sinusoids according to Popper and Schaffner) leading to increased diffusion barriers around hepatocytes and stenosis of sinusoidal blood stream. The gross rearrangement of extracellular matrix in fibrosis, as in the subendothelial space where normally a gradient in matrix chemistry from the portal triad (stem cell compartment) to the central vein is believed to exist,\(^5\) probably results in changes in gene expression, responses to hormones, growth factors, cytokines, and chemokines in those (sinusoidal) cells anchored in the mesh work of matrix molecules.\(^6\)

Activities of retinoid (fat) storing cells

Based on a large number of immunocytochemical and in situ hybridisation studies and on cell culture experiments made possible by the pioneering work of Knook et al\(^7\) and Friedman et al\(^8\) in isolating animal and human sinusoidal cell types the perisinusoidal lipocytes, synonymously termed Ito cells, fat storing, and retinoid storing cells have been identified as the precursor (see later) cell type being of importance for matrix production in damaged liver tissue.\(^9\) Perisinusoidal lipocytes, which display a low mitotic activity, are present in a ratio of 3-6-6 cells/100 hepatocytes (Ito cell index). The body of these liver specific pericytes, originally described by von Kupffer in 1876, is located in recesses of adjacent hepatocytes having dendritic cytoplasmic processes (‘stellate cells’) embracing the endothelial boundary of the sinusoid. In large triacylglycerol rich droplets more than 80% of total liver vitamin A (retinyl esters) are stored. This is delivered to these cells by secretion from hepatocytes taking up chylomikron remnant retinyl esters.\(^10\) There are preliminary indications of intralobular (zonal) heterogeneity of lipocytes with regard to vitamin A content, size of droplets, arborisation, and desmin expression, which may result from the subendothelial matrix gradient as mentioned before.\(^3\)

After the concept of ‘streaming liver’ the heterogeneity may be dynamic during the life span of a lipocyte.\(^11\) Recently, clonal heterogeneity of perisinusoidal lipocytes in cirrhotic (rat) liver has been described.\(^12\) As each clone expresses a unique phenotype, this important finding might be relevant at least partially for the variability of the fibrogenic response seen in (alcoholic) liver disease. The pathogenetic relevance of perisinusoidal lipocytes relies on their ability to be ‘activated’ in areas of necroinflammation. This process, now recognised as the key pathogenetic event in the initiation of fibrogenesis, includes (a) stimulation of proliferation, (b) phenotypic transformation (via so called transitional cells) from the ‘storing’ to the ‘synthetic’ phenotype (myofibroblast) containing much less vitamin A (droplets), hypertrophied endoplasmic reticulum and abundant desmin and smooth muscle α actin filaments, (c) gene expression and secretion of an array of matrix proteins, glycoconjugates, and glucosaminoglycans (hyaluronan), and (d) the acquisition of contractility, for example, in response to endothelin 1, also suggesting a vasoregulatory function of activated perisinusoidal lipocytes during liver injury. Activation is (spontaneously) mimicked by culturing these cells on plain plastic surfaces but certain extracellular matrix molecules used as cell support either completely block spontaneous transformation or modulate strongly gene expression.\(^13\) In addition to highly stimulated matrix synthesis perisinusoidal lipocytes change during activation, the quality and quantity of cell surface receptors (for example, platelet derived growth factor, insulin like growth factor 1 and 2, fibroblast growth factor), express a number of important

---

Leading articles express the views of the author and not those of the editor and the editorial board.

---

Gut 1994; 35: 1331–1333
cytokines (transforming growth factor (TGF) α, TGF β1, fibroblast growth factor, monocyte chemotactic peptide 1, endothelin 1, platelet activating factor, colony stimulating factor 1, insulin like growth factor 1), and secrete tissue inhibitor of metalloproteinases 1 with the ability of slowing down the degradation of newly formed collagens.14 Thus, evidence is mounting that activated perisinusoidal lipocytes are an important proinflammatory cell type having significant functions beyond the mere production of all relevant matrix molecules. Compared with activated perisinusoidal lipocytes, parenchymal liver cells, sinusoidal endothelial cells, and Kupffer cells do not participate directly and significantly in matrix production, although controversial data are reported with regard to hepatocytes.15

Paracrine and autocrine growth regulators

What are the driving forces of perisinusoidal lipocytes activation? This question tackles the epicentre of research in fibrogenesis. The approach should include not only the search for mitogenic and matrix stimulating factors but also the identification of those regulators and mechanisms keeping perisinusoidal lipocytes quiescently in normal liver. Based on current knowledge, I propose a ‘three step cascade model of perisinusoidal lipocytes activation’ implying the sequential cross talk between perisinusoidal lipocytes and hepatocytes, Kupffer cells, thrombocytes, endothelial cells, and myofibroblasts (transformed perisinusoidal lipocytes) (Figure). Accordingly, in a preinflammatory phase (A) resulting from damage of hepatocytes (probably the initiating event) synthesis of a paracrine acting, natural inhibitor of perisinusoidal lipocytes activation is reduced and renders perisinusoidal lipocytes susceptible to a broad array of cytokines and growth factors expressed during the inflammatory phase (B) in activated Kupffer cells/macrophages (TGF β, TGF α, ‘lipocyte activating factor’, etc) and released by disseminated platelets (TGF β, epidermal growth factor like factors, platelet derived growth factor, etc) at the site of necrosis. TGF β, the prototype of a fibrogenic cytokine,16 affects potently the transformation of perisinusoidal lipocytes to myofibroblasts. This last cell type is stimulated during the postinflammatory phase (C) via an autocrine loop by TGF α, TGF β, and fibroblast growth factor. Together with further paracrine stimulation of untransformed perisinusoidal lipocytes by myofibroblasts, the postinflammatory phase potentially contributes to self perpetuation of fibrogenesis even after cessation of the initiating event.17 In addition to polypeptide mediators certain low molecular weight chemical compounds like acetaldehyde, reactive oxygen species, and lactate potentially participate in perisinusoidal lipocytes activation.

Modulation by extracellular matrix and scavengers

The activity of cytokines and growth factors can be modified, both inhibited and potentiated, by interacting with the extracellular matrix. Decorin, a small proteoglycan, was shown to bind (via the core protein) TGF β neutralising its biological activity.18 As TGF β increases the expression of decorin in perisinusoidal lipocytes a negative feedback regulatory system is proposed. Another proteoglycan, soluble betaglycan (type III TGF β receptor), might also neutralise TGF β activities. As well as proteoglycans, collagens also immobilise cytokines efficiently, thus providing a potential storage site with consecutive sustained and site directed release.19 Extracellular matrix, especially in fibrotic conditions, turns out to be a sponge for growth factors. TGF β (and some other cytokines) are avidly bound to α2 macroglobulin secreted not only by hepatocytes but also by activated perisinusoidal lipocytes.20 The protease inhibitor scavenges the cytokine because the complex is internalised efficiently by the low density lipoprotein (LDL) receptor related protein/α2 macroglobulin receptor of myofibroblasts. TGF β,
which is secreted mostly in a latent form, is activated extracellularly by proteases among which the plasmin-plasminogen activator plays an important part. Stimulatory actions on cytokine function are seen for certain membrane heparan sulphate proteoglycans (betaglycans or syndecans) functioning as coreceptors for TGF β and fibroblast growth factor, respectively. Finally, the effects of cytokines on perisinusoidal lipocyte functions critically depend on the combination (or sequence) of factors and the stage of perisinusoidal lipocyte transformation. Although a comparable and reproducible staging of perisinusoidal lipocyte transformation is not yet possible because of lack of specific markers (an important cause of conflicting results between various studies), current data clearly point to dramatically changing patterns of receptor expression during transformation. Keeping in mind all these variables of cytokine function, it is quite reasonable that mere monitoring of cytokine mRNA activities and their immunohistochemical detection do not contribute importantly to the understanding of their regulation and functional significance in pathological conditions.

Therapeutic possibilities

Clearly, detailed insights into the cascade mechanism of perisinusoidal lipocyte activation and myofibroblast perpetuation (propagation) will culminate in approaches to more specific, effective, less harmful modes of treatment. In comparison with inhibition of collagen synthesis, a somewhat ancient and pathogenetically quite distant point of interference, suppression of perisinusoidal lipocyte activation and inactivation of myofibroblasts would provide a very direct therapeutic access localised at an early stage of the pathogenetic sequence. Beside the protection of hepatocytes against damage (for example, by dimethyl prostaglandin E₂), drug targeting to perisinusoidal lipocytes and myofibroblasts would be feasible but this is presently not realistic because cell type specific entry mechanisms are not understood. Modulation of perisinusoidal lipocyte receptor expression (for example, for platelet derived growth factor by pentoxifylline), application of natural receptor antagonists (for example, for interleukin 1), neutralising antibodies (for example, for TGF β), and of matrix molecules or their fragments binding (neutralising) growth factors (see above) are of great potential. In vivo application of decorin was shown in a model of experimental glomerulonephritis to inhibit increased production of extracellular matrix and to attenuate the severity of the disease. Natural inhibitors like decorin may eventually prove useful in diseases caused by overproduction of TGF β. Lipocyte activation (proliferation, matrix gene expression) in culture can be effectively inhibited by γ interferon, dexamethasone, and retinoic acid. The response of perisinusoidal lipocytes to these and other inhibitors may change during transformation as shown recently for retinoids. The nuclear retinoic acid receptor β decreases strongly during perisinusoidal lipocyte transition, which renders transformed cells less sensitive or even unresponsive to retinoids. Antithrombotic effects can also be achieved by stimulating lipocyte collagenase activity through polyunsaturated lecithin, which selectively prevents acetaldehyde induced increase in collagen accumulation in perisinusoidal lipocyte cultures and in a baboon model of alcoholic cirrhosis.

In summary, many of the interrelations discussed here for hepatic fibrogenesis concern fibrotic reactions in general – that is, they are operating in fibrotic lesions in various non-hepatic organs and atherosclerosis in a similar way. Further insight into the uniformity of fibrotic tissue reactions based on the interplay of cytokines, parenchymal and non-parenchymal cells, and the matrix requires the innovation of experimental designs that provide better images of the in situ cross talk than conventional techniques used presently. In this context, the characterisation of transforming perisinusoidal lipocytes has to be thoroughly established and refinement of cell culture techniques providing double and triple cultures and a systematic approach to ‘semi in vivo’ models permitting multidirectional instead of unidirectional studies of cell communication have to be developed. It is now a very exciting period for studying fibrogenesis, promising breakthroughs in biochemical diagnosis and effective treatment in the not too distant future.

A M GRESSNER

Department of Clinical Chemistry and Central Laboratory, Philipps University, Baldingerstrasse, 35033 Marburg, Germany

Perisinusoidal lipocytes and fibrogenesis.

A M Gressner

Gut 1994 35: 1331-1333
doi: 10.1136/gut.35.10.1331

Updated information and services can be found at:
http://gut.bmj.com/content/35/10/1331.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/