IL-12 receptor β1 (IL12Rβ1) subunit. IL-23 promotes the development of a pathogenic T lymphocyte population described as T_H-17, distinct from T_H1 and T_H2, which is characterized by IL-6, IL-17, and TNF production.[23] Therefore, it is possible that antagonism of IFN-γ signalling may have a more restricted effect compared with antagonism of IL-12p40 (antagonising both IL-12 and IL-23) and antagonism of TNF (fig 2). In addition, blockade of IFN-γ may enhance the development of T_H17 effector cells. Chronic inflammation is a nexus of pathways, and multipoint blockade may be necessary to increase clinical efficacy. Further clinical trial experience with anti-IL-12p40 and anti-IFN-γ is necessary to determine which one of these monoclonal antibodies prove to be more effective in the treatment of inflammatory bowel disease, including CD and UC.

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Liver disease

Human hepatic stellate cells are resistant to apoptosis: implications for human fibrogenic liver disease

N Kawada

Resistance of hepatic stellate cells or myofibroblasts to proapoptotic stimuli is different between rodent and human cells. This may be important when looking for antifibrotic agents that can be used in human liver fibrosis

Hepatic stellate cells (HSCs) are one of the sinusoid constituent cells that play multiple roles in liver pathophysiology and, in particular, in liver fibrosis. In the intact liver, HSCs localise in the space between sinusoids and hepatocytes, so called space of Disse, embrace the sinusoids as liver specific pericytes to regulate sinusoidal blood flow by their contractility, and store lipid droplets largely containing vitamin A.[1] When the liver parenchyma suffers from chronic injury caused by various disease aetiologies, such as iron overload, alcohol consumption, infection by hepatitis B virus or hepatitis C virus (HCV), non-alcoholic steatohepatitis, autoimmune hepatitis, and bile duct obstruction, elimination of damaged hepatocytes causes HSCs to depart from the sinusoidal wall and become activated. This process is considered to be triggered by multiple peptide, lipid, and gaseous mediators that are released from hepatocytes, Kupffer cells, endothelial cells, and infiltrating inflammatory cells.[2–5]

HSC activation accompanies their phenotypic transformation into myofibroblast (MFB)-like cells. The latter cell type exhibits expression of α smooth muscle actin and growth factor receptors, such as platelet derived growth factor receptor β (PDGF receptor β), production of contractile mediators, such as endothelin-1, and mitogenic mediators, such as PDGF, insulin-like growth factor, vascular endothelial growth factor, and chemokines, and production of extracellular matrix materials (that is, collagens, fibronectin, laminin, and proteoglycans), thereby playing major roles in the progression of fibrosis in chronically damaged livers. HSC activation is supported particularly by transforming growth factor β (TGF-β). Activated HSCs produce TGF-β1 which

promotes and maintains their own collagen gene expression in an autocrine loop. TGF-β1 also upregulates tissue inhibitor of metalloproteinases (TIMPs) which inhibit metalloproteinases and exhibit an antiapoptotic effect on HSC.[6 7]

Several recent reports have indicated that hepatic fibrosis and even cirrhosis may regress.[8–10] These observations have toppled the established theory that cirrhosis is an incurable liver disease, particularly from a pathological point of view, and increased enthusiasm for developing antifibrogenic therapies. In experimentally induced liver fibrosis in rodents, cessation of liver injury, for instance, by stopping hepatotoxin administration, results in fibrosis regression, usually mediated by reduction of TIMP-1 and apoptosis of the HSC lineage.[11] In humans, spontaneous resolution of liver fibrosis can occur after successful treatment of the underlying disease. In particular, chronic HCV infection has been most extensively studied and interferon therapy with viral eradication results in fibrosis improvement although the precise cellular and molecular mechanisms have remained unsolved.[12] Mass level regression of liver fibrosis is logically supported by experimental evidence showing that rodent HSCs/MFBs undergo apoptosis in culture. Recent studies indicate (in most cases using rat cells) that HSCs in culture undergo apoptosis via pentapeptide GRGDS (Gly-Arg-Gly-Asp-Ser), recombinant matrix metalloproteinase 9, an antibody against focal adhesion kinase, Fas/Fas ligand, nerve growth factor (NGF), tumour necrosis factor α (TNF-α), insulin-like growth factor 1, interferon γ, selective ligands for peripheral benzodiazepine receptors, high dose sphingosine-1-phosphate, gliotoxin, adenoviral overexpression of p53 or retinoblastoma protein, and so on.[13 14] However, the apoptotic characteristics of human activated HSCs/MFBs have not been fully elucidated.

Apoptosis is triggered by intrinsic and extrinsic stimuli and is mediated by the caspase cascade.[15 16] There are 13 caspases in humans. Caspases 3, 6, 7, 8, 9, and 10 are involved in cellular apoptosis. They are further divided into initiator caspase (caspases 8 and 9) and executor caspase (caspases 3, 6, and 7). Initiator caspases 8 and 9 are activated by the intrinsic pathway triggered by anticancer drugs, antioxidants, and deprivation of growth factors or serum, and can be blocked by the oncogene Bcl-2. Bcl-2 homologue 3 only proteins, such as Puma, Noxa, and Bad, stimulate mitochondria to release cytochrome c, leading to activation of caspase 9 together with apoptotic protease activating factor 1. The extrinsic pathway of apoptosis is triggered by death factors, such as Fas ligand (CD95 ligand), TNF, and TNF related apoptosis inducing ligand.[17 18] The death inducing signalling complex, consisting of a receptor, adaptor, and procaspase 8, is formed downstream of the death receptor, where procaspase 8 is autocatalytically processed and then directly activates caspase 3. Caspases 3, 6, and 7 cleave several nuclear and cytoplasmic proteins, resulting in cell death by inducing morphological and biochemical changes characteristic of apoptosis. Caspases 1, 4, 5, 11, 12, and 14 are known to be involved in the inflammatory reaction.

In this issue of *Gut*, Novo and colleagues[19] demonstrated that fully activated human HSCs/MFBs do not undergo spontaneous apoptosis and survive to prolonged serum deprivation, exposure to Fas ligand, NGF, TNF-α, doxorubicin, etoposide, and oxidative stress mediators such as hydrogen peroxide, superoxide anion, and 4-hydroxynonenal ***(see page 1174)***. Induction of caspase dependent, mitochondria driven apoptosis in human HSCs/MFBs was observed only when actinomycin D or cyclohexamide was added to the culture, indicating some protein expression contributes to the HSC/MFB resistance to apoptotic stimuli. The authors showed evidence that Bcl-2 leads to human HSC/MFB resistant to apoptotic stimuli as Bcl-2 is overexpressed in them. This did not occur in freshly isolated human HSCs, and Bcl-2 silenced cells (using the siRNA technique) became susceptible to TNF-α induced apoptosis. Furthermore, the authors demonstrated, using immunohistochemistry, that Bcl-2 staining was present in myofibroblast-like cells in areas localised at the interface between fibrotic septa and the parenchyma of cirrhotic nodules.

The results presented here raise an important clinical concern. As described above, liver fibrosis is reversible after eradication of pathogens and hepatotoxin, presumably through apoptosis of HSCs and MFBs in rodents. However, this article provides evidence that human liver fibrosis/cirrhosis would resist regression compared with rodent experimental liver fibrosis as human MFBs become fully resistant to apoptotic stimuli after a long inflammatory reaction and repeated cell replication. In this respect, in order to achieve complete resolution of human liver fibrosis, in particular cirrhosis, a novel strategy is required for induction of apoptosis of activated HSCs/MFBs in humans. Drugs that suppress Bcl-2 expression solely in human MFBs are eagerly awaited for this purpose.

In conclusion, resistance of HSCs or MFBs to proapoptotic stimuli is different between rodent and human cells. This is important to bear in mind when searching for antifibrotic agents that can be used in human liver fibrosis. Bcl-2 could be one of the targets leading to HSC/MFB sensitivity to apoptosis.

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