



B cells: no longer bystanders in liver fibrosis

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Cytokines secreted by cells that mediate the innate and adaptive immune responses play a critical role in regulating the synthesis of ECM components by fibroblasts. Overexpression and deposition of ECM components are dominant features of fibrotic diseases, including hepatic fibrosis. The contribution of CD4⁺ Th2 cells to hepatic fibrosis has been well described. Now, in this issue of the *JCI*, Novobrantseva et al. provide data to suggest that hepatic B cells also play a role in liver injury (see the related article beginning on page 3072). In a carbon tetrachloride–induced mouse model of hepatic fibrosis, T cell–deficient mice developed severe liver fibrosis; however, in B cell–deficient animals, hepatic fibrosis was attenuated. This study provides new insight into our understanding of the cells involved in mediating the adaptive immune response that leads to hepatic fibrosis.

The ECM is composed of a variety of molecules including the fibrous proteins collagen and fibrillin, adhesion molecules, glycoproteins, proteoglycans, and metalloproteinases, which together form a scaffold on which cells adhere, grow, and differentiate. The most abundant proteins in the ECM belong to the collagen family. In general, ECM molecules are synthesized by fibroblasts; in the liver, they are synthesized by activated hepatic stellate cells (HSCs), portal fibroblasts, and myofibroblasts of bone marrow origin.

Maintenance of the normal structure and function of the ECM involves constant remodeling, which results from regulated, low levels of ECM synthesis and degradation. During wound healing, there is a short-lived shift in the balance of synthesis and degradation, which leads to a transient increase in collagen production and deposition. Chronic collagen production can result in keloid scars.

A permanent imbalance resulting in major alterations in both the quantity and composition of the ECM causes fibrosis. Excessive accumulation of type I collagen is a major pathological feature in diseases such as scleroderma, osteogenesis imperfecta, and scurvy as well as other conditions of the lung, kidney, and liver characterized by tissue fibrosis (1). The main

causes of liver fibrosis include chronic HCV infection, alcohol abuse, and non-alcoholic steatohepatitis. We note that exposure to carbon tetrachloride (CCl₄), the most commonly used model of chemically induced hepatic fibrosis in rodents, is not considered to be a main cause of hepatic fibrosis in humans.

The cellular and molecular mechanisms underlying fibrotic disorders are similar regardless of the tissue involved. First, excessive collagen production results from the increased expression of the collagen gene. Second, decreased activity of ECM-removing metalloproteinases, primarily due to the overexpression of tissue inhibitors of metalloproteinases (TIMPs), results in decreased ECM (including collagen) degradation. Third, acquisition of a novel phenotype by fibroblasts is related to abnormal activation of receptors and signaling pathways (2, 3), leading to autocrine synthesis of cytokines or growth factors. This finding is supported by the observation that explanted fibroblasts from scleroderma patients retain the new phenotype during multiple serial passages in vitro. Similarly, microarray analyses have revealed high levels of expression of fibrogenic genes, which correlate with prolonged histopathological alteration in a CCl₄-induced rat model of liver fibrosis (4).

Fibrosis: an immune-driven process

Fibroblasts bear receptors that bind various growth factors and cytokines; this triggers specific signaling pathways, leading to activation of transcription factors that bind DNA elements on specific promot-

ers and subsequently regulate the expression of genes encoding ECM proteins. The majority of cytokines regulating gene expression in fibroblasts are produced by cells mediating innate or adaptive immunity (Figure 1). Cytokines and growth factors modulating fibroblast function may be grouped into 2 categories: profibrogenic cytokines (e.g., TGF-β1, IL-4, IL-13, and IL-6) and growth factors such as PDGF and connective tissue growth factor (CTGF), which are able to increase the synthesis of ECM proteins including collagen. In contrast, proinflammatory cytokines (TNF-α, IFN-γ, and IFN-α) inhibit collagen gene expression.

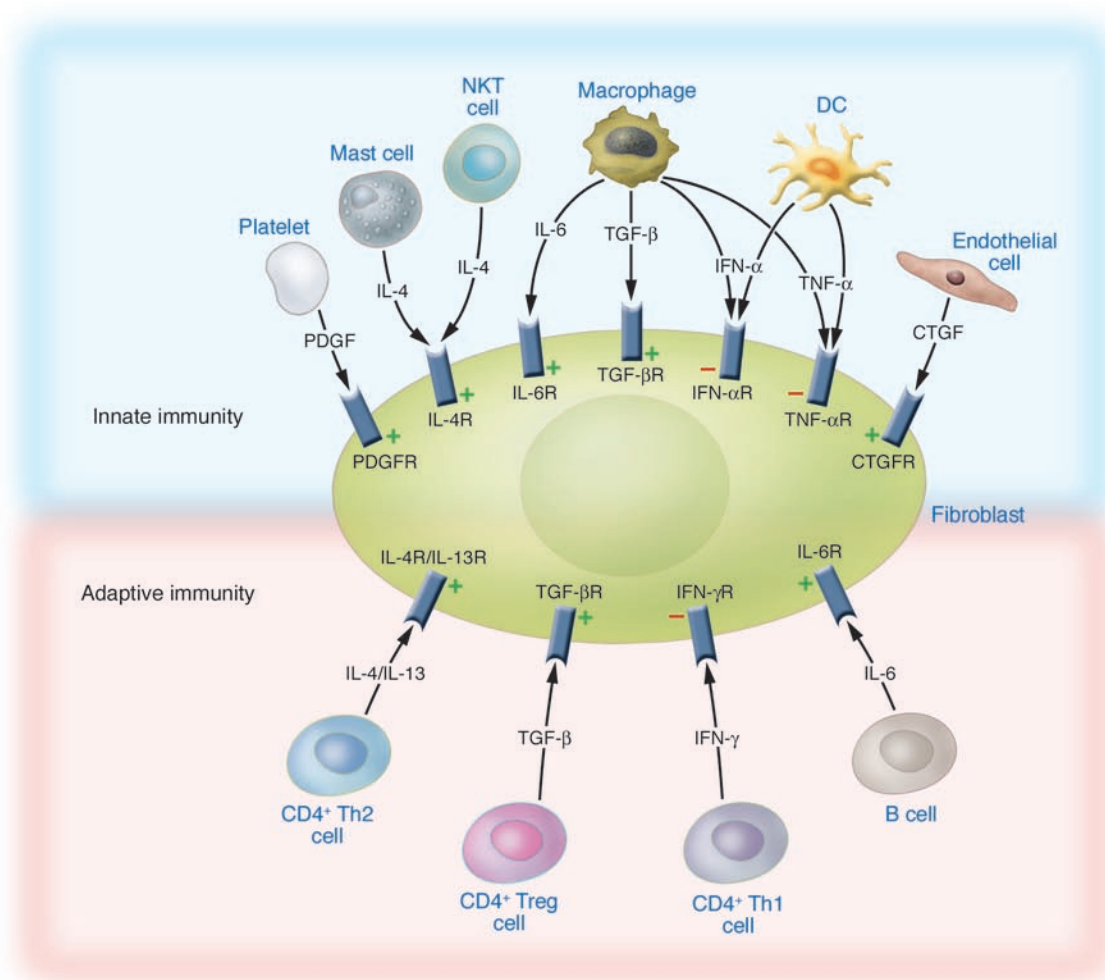
TGF-β1 is a key mediator of fibrogenesis in various tissues by virtue of its ability to increase the synthesis of ECM proteins and regulate the autocrine synthesis of PDGF and CTGF while it is also able to decrease the synthesis of metalloproteinases by increasing the synthesis of their specific inhibitors (TIMPs) (3, 5, 6). TGF-β1 plays a pivotal role in the evolution of liver fibrosis. Overexpression of the gene encoding TGF-β1 in transgenic mice enhances and causes a marked persistence of chemically induced fibrosis (7). Meanwhile, interruption of TGF-β1 signaling by delivery of a transgene encoding a dominant-negative, truncated form of TGF-β receptor II causes a dramatic reduction of dimethylnitrosamine-induced liver fibrosis in rats (8).

IL-4 and IL-13 secreted by CD4⁺ Th2 cells, NKT cells, and mast cells stimulate collagen production subsequent to their binding to corresponding receptors on fibroblasts (Figure 1). The role of CD4⁺ Th2 cell- and STAT6-mediated signaling in the development of fibrosis is well documented in studies performed in animal models, such as in the tight skin (TSK) mouse. TSK mice develop a scleroderma-like syndrome in which cutaneous fibrosis is due to a mutation in the gene encoding fibrillin-1 (9). Targeted mutation of CD4, STAT6, IL-4Rα, and IL-4 can prevent the occurrence of cutaneous fibrosis (10). Furthermore, an inhibitor of IL-13 has been shown to block the development of hepatic fibrosis (11). Therefore, Th2 cytokine-dominated

Nonstandard abbreviations used: CCl₄, carbon tetrachloride; CTGF, connective tissue growth factor; HSC, hepatic stellate cell; IH, intrahepatic; TIMP, tissue inhibitor of metalloproteinase; TSK, tight skin.

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**Figure 1**

Effects of cytokines produced by cells mediating innate and adaptive immune responses on the expression of collagen genes in fibroblasts. R, receptor.

responses play a crucial role in the pathogenesis of a variety of fibrotic diseases.

The role of B cells in the pathogenesis of TSK syndrome has not yet been elucidated. This notion is supported by the following observations: (a) TSK mice unable to produce Igs (TSK^{+/+}JD^{-/-}) do not make autoantibodies but develop cutaneous hyperplasia (12). (b) While the adoptive transfer of TSK B cells into MHC matched normal mice induces the synthesis of autoantibodies, transferred TSK B cells do not cause skin sclerosis (13). (c) Moreover, the deletion of CD19, a critical B cell surface-signaling molecule, in TSK mice abolishes the production of autoantibodies, displaying a minimal effect on skin sclerosis (14). Therefore, although B cells may not solely cause cutaneous hyperplasia, they may contribute to its development via interacting with other factors.

Various members of the PDGF family also play a role in wound healing and fibrosis. PDGF-BB is a potent mitogen for fibroblasts, including HSCs, and induces the synthesis of ECM proteins. Furthermore, steatosis and fibrosis in PDGF-C transgenic mice have been shown to be preceded by activation and proliferation of HSCs (15).

B cells and fibrotic diseases

Autoimmune diseases are classified into systemic and organ-specific diseases. Organ-specific diseases are caused by pathogenic autoantibodies specific for an epitope borne by self antigens expressed by a single cell type. The pathogenic autoantibodies produced by the B2 subset of B cells are defined by several criteria. The most faithful of these criteria is the induction of tissue damage or disease fol-

lowing experimental or maternal transfer of these autoantibodies and their isolation from target tissues (16). In systemic autoimmune diseases, autoantibodies are directed against a multitude of self antigens, but only a few fulfill the criteria of pathogenic autoantibodies. For example, anti-RO antibodies cause neonatal systemic lupus erythematosus in humans (16), and passive transfer of serum from K/BxN TCR mice to healthy animals induces many of the features of human rheumatoid arthritis (17). The role of B cells in autoimmune disease is not limited to the production of autoantibodies since B cells are also involved in antigen presentation and the production of various cytokines.

In this issue of the *JCI*, Novobrantseva et al. (18) report that B cells play a role in CCl₄-induced hepatic fibrosis in mice



in an antibody- and T cell-independent manner. The authors demonstrate that approximately half of the murine intrahepatic (IH) lymphocyte population comprised functionally operational B cells. Analyses of the VDJ rearrangements of adult murine IH B cells revealed extensive nontemplated (N,P) nucleotide additions in contrast to the few insertions observed in neonatal B cells, strongly suggesting that liver B cells belong to the B2 subset and represent a true IH B cell population. CCl₄ stimulates an inflammatory response that includes a TGF- β -induced increase in type I collagen production (7, 8). To examine the role of B cells in liver injury and repair, Novobrantseva et al. studied B cell-deficient, CCl₄-treated mice and found that collagen deposition in these mice was decreased in comparison with CCl₄-treated WT BALB/c mice. B cell-deficient mice were also able to clear apoptotic cells faster than WT animals. Hence, it was presumed that in the absence of B cells, the ability of macrophages to clear expiring hepatocytes was enhanced. As T cell-deficient mice showed similar levels of collagen deposition when compared with their WT counterparts, the role of T cells in CCl₄-induced liver fibrosis was not considered to be imperative. Finally, the authors examined whether B cell regulation of hepatic fibrosis required Ig production. Mice with normal B cell numbers that lack Ig in their serum or have low Ig levels were found to develop a similar degree of CCl₄-induced liver fibrosis when compared with WT mice, indicating that B cells have an impact on the development of liver fibrosis in an antibody-independent manner in this disease model.

The mechanism of B cell involvement in liver fibrosis remains elusive

There may be several mechanisms by which B cells have an impact on liver fibrosis. First, B cells produce the profibrotic cytokine IL-6, which may contribute to liver fibrosis by its ability to induce the differentiation of HSCs into myofibroblasts, induce fibroblast proliferation, and increase collagen and TIMP synthesis (19). IL-6 levels are increased in human chronic hepatic diseases as well as CCl₄-induced hepatic fibrosis (19). As suggested by Novobrantseva et al. (18), chronic administration of CCl₄ induces substantial necrosis/apoptosis leading

to accumulation of macrophages that remove dying hepatocytes and stimulate fibroblast function. Activated macrophages and fibroblasts could contribute to increased IL-6 synthesis, thereby increasing collagen synthesis. This is in agreement with the observation that chronic administration of IL-6 induces hepatic inflammation, increased synthesis of collagen, and fibrosis (20). The fact that CCl₄-mediated necrosis and apoptosis in mice is actually reduced in IL-6-deficient mice would support the notion that IL-6 promotes fibrosis (21). This effect may be due to downregulation of MMP2, a metalloproteinase that mediates collagen degradation (22).

Second, alterations in T cell function in B cell-deficient mice may contribute to the observed impact of B cells on liver fibrosis. Progressive CCl₄-induced liver fibrosis in BALB/c mice is associated with increased levels of IL-4 and decreased levels of IFN- γ , synthesized by CD4⁺ Th2 and CD4⁺ Th1 cells, respectively (23). As described above, these cytokines have a profound regulatory role in collagen gene expression. Therefore, it is not out of the question that dysfunction of CD4⁺ T cells in B cell-deficient mice may contribute to attenuated progression of CCl₄-induced fibrosis.

Finally, another plausible explanation for the attenuated fibrosis observed in B cell-deficient mice is that the lack of autoantibody production may affect profibrogenic cytokine activity. It has been demonstrated that anti-fibrillin autoantibodies are present in TSK mice and scleroderma patients (24). Fibrillin-1 is localized in the walls of portal connective tissue, and its expression is increased during fibrosis (25). Therefore, one may consider that anti-fibrillin autoantibodies are present and play a role in liver fibrosis. It was recently shown that anti-fibrillin-1 autoantibodies from scleroderma patients may play a pathogenic role by increasing the transcription and production of ECM components in normal human fibroblasts via the release of sequestered TGF- β 1 from fibrillin-1 (26).

In conclusion, the study by Novobrantseva et al. (18) offers insight into the mechanisms driving hepatic fibrosis, particularly regarding the contribution of B cells to liver tissue injury and repair.

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CD36 may determine our desire for dietary fats

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There is a strong link between high fat intake and obesity. In addition to its high caloric density, dietary fat has a hyperphagic effect, in part as a result of its high palatability. The recent identification by Laugerette et al. of CD36 as a taste receptor for fatty acids provides insight into the molecular basis of our preference for fat (see the related article beginning on page 3177). As we gain more information regarding the function of this receptor, we may be able to devise better strategies to address the addictive potential of dietary fat.

The eighteenth-century French philosopher Charles De Montesquieu once commented, “Lunch kills half of Paris, supper the other half.” The potential of food consumption to lead to serious health complications is well known and has been extensively studied. The last decade has witnessed impressive progress related to some of the molecular mechanisms underlying the development of nutrition-related pathologies such as obesity, type 2 diabetes, and cardiovascular disease. Information on interorgan cross-talk, on various adipokines and myokines, and on proteins involved in controlling energy intake, storage, or expenditure has greatly enhanced our insight into how the body maintains homeostasis (1, 2). Dysfunction within 1 or more homeostatic mechanisms can occur as a result of complex interactions between genetic predisposition and today's affluent lifestyle, often leading to serious health consequences. This has been highlighted by numerous studies of genetically altered animals and by the identification of polymorphisms in humans (3–5).

A potential new frontier in nutrition research is the examination of how the orosensory experience of food can impact food intake and processing as well as the development of long-term addictive patterns. In addition to food abundance, current enhancements in food palatability through high sugar or fat content further challenge our ability to control food intake and maintain body weight homeostasis. The sensory experience of food can be a primary reinforcer of intake. To what extent food perception is determined by genes versus the environment is a topic that has received limited attention. There is evidence that obesity may be associated with an abnormal brain response to the sensory perception of a meal. This abnormal response may even persist in post-obese individuals, creating a high risk of relapse (6). There is little doubt that as individuals, we greatly differ in our ability to experience food at the basic level of taste. According to the NIH, approximately 25% of Americans are nontasters, 50% are medium tasters, and 25% are supertasters. So what are the factors that contribute to determining our food perception, and how are they reflected in what we choose to eat and how much? These questions are important, since our food choices greatly impact body weight outcome in terms of how big and how fast. Impressive progress

has been recently accomplished in the identification of taste receptors for various sensations such as sweet, salty, and bitter (7), and this is beginning to contribute insight into the interaction between heredity and the environment in determining food preferences and intake patterns (8, 9).

Why do we like fat?

An exciting new development is the identification of a taste receptor for fat that specifically recognizes fatty acids (FAs), as reported in this issue of the *JCI* by Laugerette et al. (10). It seems a propos that the report is by a group of French researchers from the University of Bourgogne in Dijon, an area with a rich gastronomic tradition. Dietary fat is particularly addictive, and its excessive intake is strongly linked to obesity. Orosensory perception is thought to play an important role in the spontaneous preference for fat-rich food exhibited by humans and rodents. A hyperphagic effect of a diet with high fat content has been documented and is manifested in increased meal size and decreased inter-meal interval (11). Postingestive effects of fat, which include feelings of contentment and satiety and possibly elevation in endogenous opiate levels, also promote long-term preference and positive reinforcement. These effects are not observed with equally palatable, but nondigestible, fat substitutes (12).

Existence of an orosensory receptor for FAs would necessitate a revision of currently held concepts related to food perception. Textbooks still state that taste buds recognize 5 basic sensations; sweet, sour, bitter, salty, and umami (L-amino acid). Evidence presented in several earlier publications strongly suggested that the

Nonstandard abbreviations used: FA, fatty acid; SR-BI, scavenger receptor type B, class I.

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