In addition, the authors showed that the proliferative response of a human B-lineage leukemic cell line to SDF-1 is PKC-ζ dependent. It remains to be seen how this relates to the mechanism by which SDF-1 influences the cycling status of primitive normal cells, since it appears that the result can range from induced quiescence (14, 15) to enhanced turnover (16), depending on the type of progenitor being assessed, the context of its exposure, and/or the concentration of SDF-1 present.

**Clinical implications**

As pointed out by Petit et al. (13), pinpointing the molecular signaling mechanisms that mediate SDF-1 effects on primitive hematopoietic cells and leukemic cells may have important implications for future therapies. The present findings certainly introduce the possibility of considering new agents for improving stem cell mobilization regimes. More speculative is the concept of exploiting small molecule inhibitors of PKC-ζ to interfere with SDF-1–promoted metastases. Since the initial report of a role of SDF-1 in breast cancer metastases (17), evidence that this pathway is hijacked in numerous other tumors has been obtained (18). The study by Petit et al. (13) has thus also set the stage for examining a new approach to the treatment of disseminating malignant cells.

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Mac the knife? Macrophages — the double-edged sword of hepatic fibrosis

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Progression of hepatic fibrosis requires sustained inflammation leading to activation of stellate cells into a fibrogenic and proliferative cell type, whereas regression is associated with stellate cell apoptosis. The contribution of hepatic macrophages to these events has been largely overlooked. However, a study in this issue of the *JCI* demonstrates that macrophages play pivotal but divergent roles, favoring ECM accumulation during ongoing injury but enhancing matrix degradation during recovery (see the related article beginning on page 56). These findings underscore the potential importance of hepatic macrophages in regulating both stellate cell biology and ECM degradation during regression of hepatic fibrosis.

Nonstandard abbreviations used: CCLs, carbon tetra-chloride; TIMP-1, tissue inhibitor of metalloproteinase-1; TRAIL, TNF-related apoptosis-inducing ligand.

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The paper by Duffield and colleagues in this issue of the JCI (4), however, firmly reestablishes the hepatic macrophage as a central determinant of the liver’s response to injury and repair. The study is built upon 2 important concepts. First, macrophages have more than one pathway of activation and response, depending on the specific stimulus and biologic context; indeed, divergence of macrophage responses has been recognized for many years (5, 6) but has only recently been placed in a biologically coherent context that defines pro- and anti-inflammatory phenotypes. Proinflammatory actions of macrophages include antigen presentation, T cell activation, and cytokine and protease release, among others (7). However, anti-inflammatory actions have been increasingly appreciated, particularly those induced by IL-4, including induction of immune tolerance (8), innate immunity (9), and T cell differentiation (7, 10).

A second, more recently developed concept addresses the behavior of hepatic stellate cells in the resolution of liver injury and fibrosis. Whereas initial interest in stellate cell biology focused on the cells’ activation and fibrogenic properties during progressive liver injury, more recent efforts have explored both their fate as liver injury resolves and the mechanisms underlying persistence of fibrosis in sustained liver injury. This is a vital area of inquiry since clarification of mechanisms by which the liver naturally restores its architecture might be exploited in developing antifibrotic therapies for patients with chronic liver disease. During regression of experimental liver fibrosis, activated stellate cells undergo programmed cell death associated with loss of tissue inhibitor of metalloproteinase–1 (TIMP-1) expression (11). Because TIMP-1 not only enhances stellate cell survival but also antagonizes matrix degradation, the loss of TIMP-1–expressing stellate cells is thought to unleash latent matrix-degrading activity, leading to the breakdown of scar matrix and the reconstitution of normal hepatic architecture. When liver injury persists, TIMP-1 levels remain high, and progressive cross-linking of collagen may render the accumulating matrix relatively insoluble to proteases (12, 13). This concept is reinforced by studies demonstrat-
ing attenuation of fibrosis when TIMP-1 is inhibited (14). Significant gaps in this paradigm have persisted, however, including the source(s) and identity of salutary proteases in fibrosis resolution and the role of other cellular elements, including not only hepatic macrophages, but also sinusoidal endothelium.

Macrophage depletion in mice identifies novel roles in regulating hepatic fibrosis

Duffield and colleagues have generated a transgenic mouse model in which macrophages can be selectively depleted in a regulated manner (4). Expression of a human version of a diptheria toxin receptor driven by the promoter of a myeloid antigen (CD11b) renders transgenic macrophages (called CD11b-DTR cells) susceptible to killing by administration of diptheria toxin. Careful control experiments confirmed that susceptibility is macrophage specific and does not affect other cell lineages. Next the investigators compared the impact of macrophage depletion on hepatic fibrosis between a murine model in which macrophages were depleted during the progression of fibrosis by the administration of diptheria toxin after 12 weeks of carbon tetrachloride (CCL4) intoxication but before the final dose of CCL4, and another in which macrophages were depleted by diptheria toxin administration only 3 days after the last dose of toxin was administered, at which time recovery from CCL4-mediated fibrosis had begun. These modest differences between the progression and recovery models of hepatic fibrosis in the timing of macrophage depletion (approximately 10 days) — specifically, the occurrence of depletion either before or after the final dose of CCL4 — yielded completely opposite effects on hepatic matrix accumulation. Depletion of macrophages while injury was progressing had an antifibrotic effect whereas depletion during early recovery led to sustained accumulation of matrix.

Divergent phenotypes of hepatic macrophages — one cell type or two?

These results are fascinating, and they raise some important but unanswered questions regarding the underlying mechanisms. Are the same macrophages responsible for promoting matrix accumulation during progression and preventing it during recovery through phenotype switching, or are different subsets of macrophages recruited during this brief interval? While the former seems more likely and is consistent with evolving concepts (7, 15), an alternative explanation also supported by this (4) and much earlier studies (16) is the recruitment and differentiation of bone marrow–derived monocytes into hepatic macrophages. In fact, it is uncertain whether all these recruited monocytes become classic Kupffer cells or remain phenotypically distinguishable as a novel subpopulation. Regardless, if phenotypic conversion of the same cells accounts for this divergent behavior in progression versus recovery, what are the signals driving this switch? One approach to clarifying these questions would be to isolate CD11b-DTR-expressing cells by flow cytometry at different intervals during progression and recovery (without diptheria toxin administration) to analyze their transcriptional profile by microarray and to perform functional studies. This approach could be used in mice chimeric for bone marrow cells so that the phenotype of CD11b-DTR–expressing cells from liver could be distinguished from those derived from bone marrow.

How do the divergent macrophage phenotypes present during fibrosis progression versus recovery affect matrix production and degradation? In particular, are the effects of macrophage depletion mediated largely through their impact on activated stellate cells and/or myofibroblasts? During fibrosis progression, it is believed that macrophages are likely to promote activation of stellate cells through release of paracrine factors, including TGF-β1, since this phenomenon has been demonstrated in culture (17, 18) (Figure 2). In contrast, the simultaneous loss of macrophages (termed scar-associated macrophages by Duffield et al.; ref. 4) and activated stellate cells during recovery suggests that macrophages could also provoke apoptosis of hepatic stellate cells by the expression of TNF-related apoptosis-inducing ligand (known as TRAIL) and other apoptotic stimuli, as recently reported (19). Thus, the biphasic nature of macrophages could yield divergent effects on stellate cells, promoting their activation during progression and apoptosis during recovery. One potential regulator of this duality could be the cytokine TNF-α or its downstream signaling mediator NF-κB, since delicate regulation of NF-κB signaling in hepatocytes can elicit profound and divergent effects on apoptosis and growth (20).

Do hepatic macrophages degrade ECM during fibrosis regression?

A more compelling question is whether macrophages directly promote matrix degradation during resolution of liver fibrosis through production of matrix-degrading proteases or by stimulating either the release or activation of proteases by other cell types, including stellate cells. Identifying macrophages as the elusive source of enzymes that degrade the fibrillar interstitial (i.e., scar) matrix during fibrosis resolution would represent a major advance, although the primary substrate for the hepatic macrophage’s major known metalloproteinase, MMP-9, is basement-membrane collagen, not fibrillar collagen (21). Still, more careful characterization of recovery-associated macrophages could yield some surprises, including macrophage release of additional proteases or the existence of a more broad substrate specificity of MMP-9 that also includes fibrillar collagens; moreover, recent data suggest that activated stellate cells may also express MMP-9 (22). Finally, degradation of fibrillar ECM by recovery-associated macrophages may yield an altered extracellular milieu that no longer supports survival of activated stellate cells, since breakdown of interstitial collagen represents loss of an important survival signal (23).

In summary, the exciting study by Duffield and colleagues (4) offers a new perspective in the study of hepatic injury and repair by resurrecting the prominence of hepatic macrophages, illustrating their dual phenotype in fibrosis progression and recovery, and highlighting their importance in regulating hepatic matrix degradation.

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Pregnane X receptor (PXR) plays an important role in detoxifying xenobiotics and drugs. In this issue of the JCI, Pascussi et al. (see the related article beginning on page 177) provide convincing evidence that PXR can also induce vitamin D deficiency and bone disease because of its ability to cross-talk with the vitamin D–responsive gene that catabolizes 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. This cross-talk behavior has important health ramifications and can be mitigated through the identification and treatment of PXR-induced vitamin D deficiency.

Historical perspective
In 1967 Schmid (1) reported an association between osteomalacia and antiepileptic drug therapy. This was confirmed by Dent et al. (2), who noted that osteomalacia and rickets were common in patients on long-term antiepileptic drug therapy, especially those who had been institutionalized and treated with multiple drugs to control their seizure disorder. Since these initial observations were made, there have been a multitude of reports of abnormalities in calcium, vitamin D, and bone metabolism in subjects chronically treated not only with antiepileptic drugs but also with glucocorticoids, rifampin, and antiretroviral drugs (3–6). The disturbances observed in antiepileptic drug–treated patients were noted to be very similar to those of patients with vitamin D deficiency. More than 50% of children and adults receiving chronic antiepileptic drug therapy are at risk for developing abnormalities in calcium, vitamin D, and bone metabolism (3). However, cardiac patients who had been treated with conventional doses of phenytoin for control of arrhythmias were found to be free of these abnormalities (7).

Antiepileptic drug–induced alterations in calcium and bone metabolism can include biochemical abnormalities such as hypocalcemia, hypophosphatemia, and elevated serum concentrations of alkaline phosphatase, parathyroid hormone, and 1,25-dihydroxyvitamin D [1,25(OH)2D]. The biochemical hallmark for this disorder is reduced serum concentration of 25-hydroxyvitamin D [25(OH)D], the major circulating form, which is a barometer for a person’s vitamin D status (8). There is an associated decrease in intestinal calcium absorption, a reduction in urinary calcium excretion, and an increase in bone turnover, as evidenced by increases in osteocalcin, bone-specific alkaline phosphatase, procollagen type I C-terminal extension peptide, and bone resorption markers including human collagen type I C-terminal peptide and total free deoxypyridinoline. The bone condition most often associated with chronic antiepileptic drug treatment is reduced bone mineral density and cortical bone loss. Rickets and, according to histologic evidence, osteomalacia indicating a defect in bone mineralization are also accompanying problems (3).

Mechanism for drug-induced disorders of bone metabolism
A variety of mechanisms have been proposed for the antiepileptic drug–induced disorders in calcium and bone metabolism. Gascon-Barré et al. (3–5) and Hahn et al. (6–9) have shown that the biochemical and histological abnormalities associated with antiepileptic drug treatment can be reproduced in experimental animal models. The biochemical and histological abnormalities associated with antiepileptic drug treatment can also be reproduced in human patients who are treated with multiple antiepileptic drugs. These findings provide strong evidence that antiepileptic drug treatment is associated with a decrease in serum concentration of 25-hydroxyvitamin D, a decrease in intestinal calcium absorption, and an increase in bone turnover, as evidenced by increases in osteocalcin, bone-specific alkaline phosphatase, procollagen type I C-terminal extension peptide, and bone resorption markers including human collagen type I C-terminal peptide and total free deoxypyridinoline. The bone condition most often associated with chronic antiepileptic drug treatment is reduced bone mineral density and cortical bone loss. Rickets and, according to histologic evidence, osteomalacia indicating a defect in bone mineralization are also accompanying problems (3).