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Edgar D. Charles, Lynn B. Dustin

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Commentary

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Chemokine antagonism in chronic hepatitis C virus infection

Edgar D. Charles and Lynn B. Dustin

Center for the Study of Hepatitis C, Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, New York, USA.

Immune responses to hepatitis C virus (HCV) fail to clear the virus in most individuals. Why patients who are less likely to clear HCV infection have high plasma levels of CXCL10 (also known as IP-10), a chemokine that directs T cells to sites of infection, has long been unclear. In this issue of the *JCI*, Casrouge and colleagues shed light on this paradox by showing that CXCL10 in the plasma of many HCV patients is enzymatically processed to produce a CXCL10 receptor antagonist. These findings introduce a role for chemokine antagonism during HCV infection and unveil new avenues for improved HCV diagnosis and therapy.

Over 120 million persons worldwide have chronic HCV infection (1), which is a major cause of liver failure and hepatocellular carcinoma (2). Up to one-quarter of persons who are acutely infected with HCV spontaneously clear their infection, and the current standard of care — pegylated IFN-α (peg–IFN-α) and ribavirin — eliminates virus in only about half of those treated (3). This means that a substantial number of patients remain chronically infected with HCV. In these chronically infected individuals, HCV-specific T cells are ineffective at eradicating virus, yet are potent mediators of hepatocellular injury. Evidence presented in this issue of the *JCI* by Casrouge et al. (4) suggests that chemokine antagonism may contribute to this inability to clear HCV infection. Their data (4) also provide an explanation as to why high levels of the chemokine CXCL10 in the plasma or serum of an HCV-infected patient portend a poor response to peg–IFN-α and ribavirin (5–8).

Salient features of chemokines

Chemokines have a central role in inflammation and host defense. These small (8–17 kDa) cytokine-like molecules act to guide leukocytes along a concentration gradient toward lymphoid organs and sites of inflammation. They also play roles in embryogenesis, angiogenesis, and lymphoid organ development. Chemokines involved in inflammation are displayed on proteoglycans near the site of their production. Chemokines bind to G protein–coupled, seven-transmembrane receptors, of which there are almost twenty. CXCR3, the CXCL10 receptor, is expressed on activated T cells, NK cells, and some B cells (9). In the hepatic sinusoid, leukocyte recognition of chemokines triggers conformational changes in the integrins that they express on their surface, which are then able to mediate binding to endothelial ligands. These steps permit leukocyte transmigration to target tissue (Figure 1) (reviewed in ref. 10).

CXCL10 and liver disease

Among chemokines, CXCL10 plays a central role in liver inflammation, and it is expressed in the HCV-infected liver (11–13). Serum CXCL10 is also elevated during flares in HBV infection (14), in primary biliary cirrhosis, and in rheumatoid arthritis (15). In several independent studies, elevated serum/plasma levels of CXCL10 predict the failure of IFN-α–based HCV treatment (5–8).

Why a chemoattractant seemingly so potent as CXCL10 is elevated in patients who fail to clear HCV has been paradoxical. One possibility is that CXCL10 is overproduced in a futile attempt to draft pusillanimous T cells into the liver to combat infection. Indeed, chronic HCV infection is often associated with impaired function and reduced breadth of continuously activated, HCV-specific T cells (reviewed in ref. 16). However, in this issue of the *JCI*, data from Casrouge and colleagues suggest that CXCL10 may in fact be dissuading T cells from joining the fight (4).

Casrouge and colleagues performed a multianalyte profiling of patient plasma, confirming that CXCL10 levels are increased in patients that do not respond to anti-HCV therapy compared with those that do (4). They also observed that CXCL10 levels correlated with elevated numbers of circulating $CXCR3^+$ cells. It had previously been proposed that the high levels of CXCL10 in patients who do not respond to anti-HCV therapy could act as an antagonist of T cell migration (5). Further, it has been reported that CXCL10 can be processed in vitro by dipeptidyl peptidase IV (DPP4; also known as CD26), which cleaves two amino acid residues from the amino terminus of CXCL10 and turns it into a CXCR3 antagonist (17), and that HCV patients have increased soluble DPP4 activity (18). However, distinguishing full-length from DPP4-processed CXCL10 in clinical samples has not been feasible until now.

After developing reagents to distinguish full-length from DPP4-processed CXCL10, Casrouge and colleagues found

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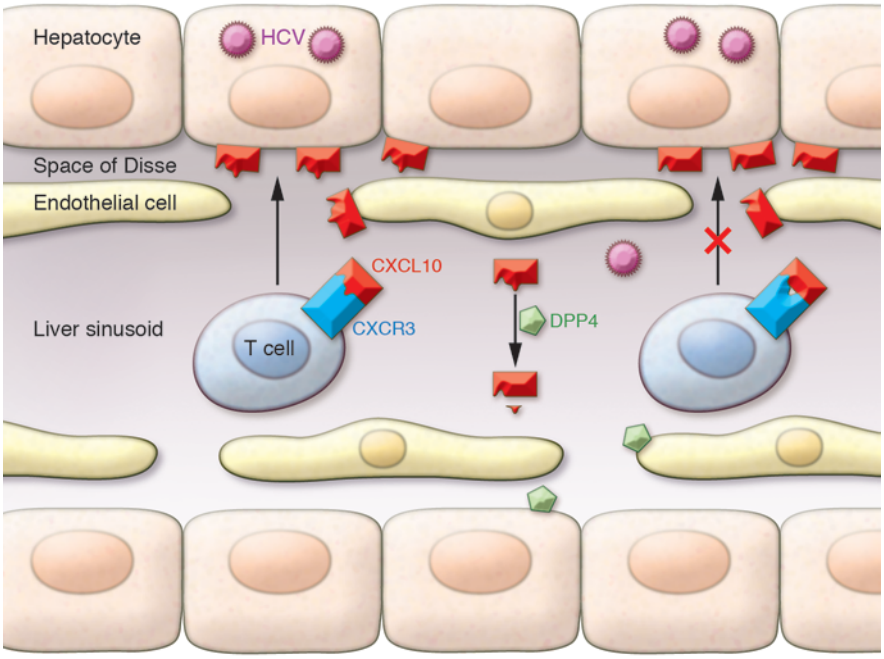

Figure 1
Model of chemokine antagonism in the HCV-infected liver. CXCL10 produced in the infected liver recruits T cells from the blood to the infected hepatocyte via the liver sinusoid and the space of Disse (left). When processed by DPP4, CXCL10 becomes an antagonist of T cell recruitment (right). In this issue of the *JCI*, Casrouge and colleagues have shown that levels of this shortened antagonist form of CXCL10 are increased in many patients who fail to clear HCV (4), suggesting a role for chemokine antagonism in an ineffective anti-HCV response.

that, in many HCV-infected patients who do not respond to therapy, circulating CXCL10 is indeed processed into the shorter form (4). Importantly, patient blood had been collected in tubes containing a DPP4 inhibitor, as DPP4 remains active after blood collection. The authors demonstrated that before treatment, plasma DPP4 activity was higher in those individuals who went on to fail to respond to anti-HCV therapy than in patients who responded and healthy individuals. They also confirmed a previous report (17) showing that CXCL10 is cleaved by DPP4 in vitro. A DPP4 inhibitor, sitagliptin — which is used clinically in the treatment of type 2 diabetes — inhibited this cleavage. Using in vitro systems, Casrouge and colleagues showed that the full-length form of CXCL10, but not the short form, could direct the migration of $CXCR3^+$ T cells (4). Short CXCL10 antagonized signaling by long CXCL10. Finally, they demonstrated that the short, antagonist form of CXCL10 predominates in the plasma of chronically infected patients who are destined to fail anti-HCV therapy; early virological responders were more likely than nonresponders to have undetectable amounts of short-form CXCL10. This latter finding will need to be confirmed in larger studies of patient cohorts carefully matched for liver function and inflammation. These results suggest that short-form CXCL10 in the plasma may antagonize T cell recruitment to the liver parenchyma (Figure 1).

Answers beget questions

The study by Casrouge et al. (4) raises new questions regarding HCV immunopathogenesis. Seemingly at odds with the study, plenty of T cells are present in the liver during HCV infection, even in those with elevated CXCL10 levels (19) and those with cirrhosis (13). It is unknown whether these T cells are all HCV-specific. One hypothesis to explain this discordance is that the short form of CXCL10 preferentially antagonizes T cells recognizing HCV peptides in an HLA-dependent context. Also, it is likely that chemotaxis of HCV-specific T cells to the liver depends upon the combinatorial effect of multiple chemokines with disparate roles and potencies. Of note, the importance of T cells in treatment-induced clearance of HCV remains controversial (16).

Importantly, the findings of Casrouge et al. (4) provide a rationale for the validation of short-form CXCL10 and DPP4 (which may be broad markers of inflammation) as clinically appropriate predictors of HCV clearance, potentially allowing for more individualized treatment decisions. It will also be useful to determine whether levels of DPP4 and short-form CXCL10 predict spontaneous resolution or chronic evolution of acute HCV infection. The successful validation of DPP4 and CXCL10 as predictors of spontaneous resolution could improve the early initiation of anti-HCV therapy in those who would most benefit, while sparing those who will clear HCV infection without treatment and those who will not respond to treatment from a therapy that has severe side effects and is therefore very poorly tolerated.

Hints about a link to diabetes?

Patients chronically infected with HCV are at increased risk of insulin resistance and frank type 2 diabetes (20, 21). Postprandial insulin secretion is controlled in part by the insulin secretagogue glucagon-like peptide–1 (GLP-1). The extremely short in vivo half-life of GLP-1 (1–2 minutes) is due to its inactivation by DPP4. The observation that DPP4 activity is increased in many HCV patients may provide clues about the pathogenesis of HCV-associated metabolic dysregulation. It is tempting to speculate that DPP4-mediated cleavage of GLP-1 may be partially responsible for HCV-related insulin resistance. Indeed, decreased levels of serum GLP-1 and increased levels of serum and liver DPP4 have been reported in HCV-infected patients relative to healthy volunteers (22). Intriguingly, levels of serum CXCL10 are also elevated in HCV patients with type 2 diabetes (23). Of course, much more work needs to be done to establish links between DPP4, CXCL10 levels, and type 2 diabetes. The next logical step is a comparison of levels of plasma DPP4 and GLP-1 in HCV-infected patients with and without impaired glucose tolerance or type 2 diabetes.

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Address correspondence to: Lynn B. Dustin, Center for the Study of Hepatitis C, Laboratory of Virology and Infectious Disease, The Rockefeller University, 1230 York Ave., Box 64, New York, New York 10065, USA. Phone: 212.327.7067; Fax: 212.327.7048; E-mail: dustinl@rockefeller.edu.

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Cytokinesis failure and attenuation: new findings in Fanconi anemia

Philip J. Mason and Monica Bessler

Division of Hematology, The Children's Hospital of Philadelphia, and University of Pennsylvania, Philadelphia, Pennsylvania, USA.

The hallmarks of the rare inherited disorder Fanconi anemia (FA) are progressive bone marrow failure and susceptibility to cancer. The former is the major cause of death for patients with FA, as it usually occurs earlier in life than cancer development. Despite spectacular advances in unraveling the molecular details of FA, the origin of the bone marrow failure that is central to this condition for most patients has long been puzzling and controversial. Two studies recently published in the *JCI*, including one in this issue, will add to the debate. They also highlight the fact that studying rare disorders can elucidate important new clinical and biological principles.

Fanconi anemia (FA) is a recessive, inherited disorder that occurs with an incidence of approximately 1 per 350,000 births, that is, relatively rarely. One of the major hallmarks of FA is bone marrow failure (BMF), which is the major caused of death in patients with FA, but it is also associated with an increased susceptibility to leukemia and certain cancers (1). FA is caused by mutations in one of fourteen or more genes (the so-called FA genes), whose products cooperate to ensure the repair of interstrand crosslinks that form during DNA replication (i.e., during the S phase of the cell cycle) (2). Failure to repair these lesions efficiently leads to the activation of S and G_2 cell cycle checkpoints that trigger cell cycle arrest (3, 4). Failure of cells, particularly HSCs and blood cell precursors, to progress through the cell cycle has been thought to contribute to the BMF experienced by patients with FA (5). Despite intensive investigation of the nature and function of the FA genes, the precise mechanism(s) underlying BMF and cancer susceptibility in FA has not been fully elucidated and remains controversial. The same can be said of other inherited BMF syndromes, such as dyskeratosis congenita, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome (6). In these diseases, BMF develops slowly over the course of many years, making the process difficult to investigate in a cell line or even in a short-lived mouse model.

Two remarkable studies recently published in the *JCI*, including one in this issue, report findings that will change the way we think about BMF in FA and may eventually change the way patients with the disease are cared for and treated (7, 8). In a recent issue of the *JCI*, Vinciguerra et al. (7) reported that some FA cells proceed past the G_2 checkpoint with DNA that is

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