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RANTES antagonism: A promising approach to treat chronic liver diseases

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COMMENTARY ON:

Antagonism of the chemokine Ccl5 ameliorates experimental liver fibrosis in mice. Marie-Luise Berres, Rory R. Koenen, Anna Rueland, Mirko Moreno Zaldivar, Daniel Heinrichs, Hacer Sahin, Petra Schmitz, Konrad L. Streetz, Thomas Berg, Nikolaus Gassler, Ralf Weiskirchen, Amanda Proudfoot, Christian Weber, Christian Trautwein, and Hermann E. Wasmuth. The Journal of Clinical Investigation 2010;120(11):4129–4140. Copyright 2010. Abstract reprinted with permission of the American Society for Clinical Investigation.

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Abstract: *Activation of hepatic stellate cells in response to chronic inflammation represents a crucial step in the development of liver fibrosis. However, the molecules involved in the interaction between immune cells and stellate cells remain obscure. Herein, we identify the chemokine CCL5 (also known as RANTES), which is induced in murine and human liver after injury, as a central mediator of this interaction. First, we showed in patients with liver fibrosis that CCL5 haplotypes and intrahepatic CCL5 mRNA expression were associated with severe liver fibrosis. Consistent with this, we detected Ccl5 mRNA and CCL5 protein in 2 mouse models of liver fibrosis, induced by either injection of carbon tetrachloride (CCl_4) or feeding on a methionine and choline–deficient (MCD) diet. In these models, $Ccl5^{-/-}$ mice exhibited decreased hepatic fibrosis, with reduced stellate cell activation and immune cell infiltration. Transplantation of Ccl5-deficient bone marrow into WT recipients attenuated liver fibrosis, identifying infiltrating hematopoietic cells as the main source of Ccl5. We then showed that treatment with the CCL5 receptor antagonist Met-CCL5 inhibited cultured stellate cell migration, proliferation, and chemokine and collagen secretion. Importantly, in vivo administration of Met-CCL5 greatly ameliorated liver fibrosis in mice and was able to accelerate fibrosis regression. Our results define a successful therapeutic approach to reduce experimental liver fibrosis by antagonizing Ccl5 receptors.*

Keywords: RANTES, Chemokines; Liver fibrosis; Hepatic stellate cells; Inflammation.
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Abbreviations: HSC, hepatic stellate cells; RANTES, regulated upon activation normal T-cell expressed and secreted; CCl_4, carbon tetrachloride; HCV, hepatitis C virus.

Most types of chronic liver disease are characterized by different degrees of hepatocellular damage, inflammatory cell infiltrate in the hepatic parenchyma, and tissue remodeling, ultimately resulting in progressive fibrosis and cirrhosis. Following repeated liver injury, a complex interplay between damaged hepatocytes, inflammatory cells, and non-parenchymal cells occurs. Infiltrating inflammatory cells at the sites of liver injury are directed to remove apoptotic cells but in addition they secrete a number of chemokines that stimulate resident cells such as hepatic stellate cells (HSCs). The resulting activated HSCs proliferate and accumulate in the injured liver, secreting large amounts of extracellular matrix proteins. Therefore, chemokines are currently considered key drivers of liver fibrogenesis and potential targets for therapy [1–3]. Chemokines are chemotactic cytokines that regulate the movement of circulating leukocytes by binding to their specific seven-transmembrane domain G-protein-coupled receptors [4]. According to the presence and position of a conserved amino-proximal cysteine-containing motif, they are classified into four subfamilies: CC, CXC, CX3C, and C chemokines [3]. CC chemokines are the largest family and are defined by the location of the first two cysteine residues in the sequence, which are adjacent. This group is known also as β-chemokines or 17q chemokine family, due to a gene cluster on human chromosome 17q11–q32 [4,5]. CCL5, also known as "regulated upon activation, normal T-cell expressed, and secreted" (RANTES), is a small CC chemokine that has powerful chemoattracting properties toward T cells, dendritic cells, eosinophils, NK cells, mast cells, and basophils. CCL5 is produced by different cell types including T cells, platelets, macrophages, endothelial cells, and fibroblasts and exerts its actions by binding to three receptors (CCR1, CCR3, and CCR5) [6]. A growing body of evidence indicates that RANTES is involved in a variety of inflammatory conditions including atherosclerosis and obesity, which share common pathophysiological pathways with liver diseases [7,8]. Pharmaceutical companies have recently developed RANTES inhibitors/CCR5 antagonists, which are currently being evaluated in several inflammatory diseases. Moreover, because CCR5 is involved in HIV entry to target cells, CCR5 antagonists have been successfully tested in phase III studies in patients with HIV infection [9].

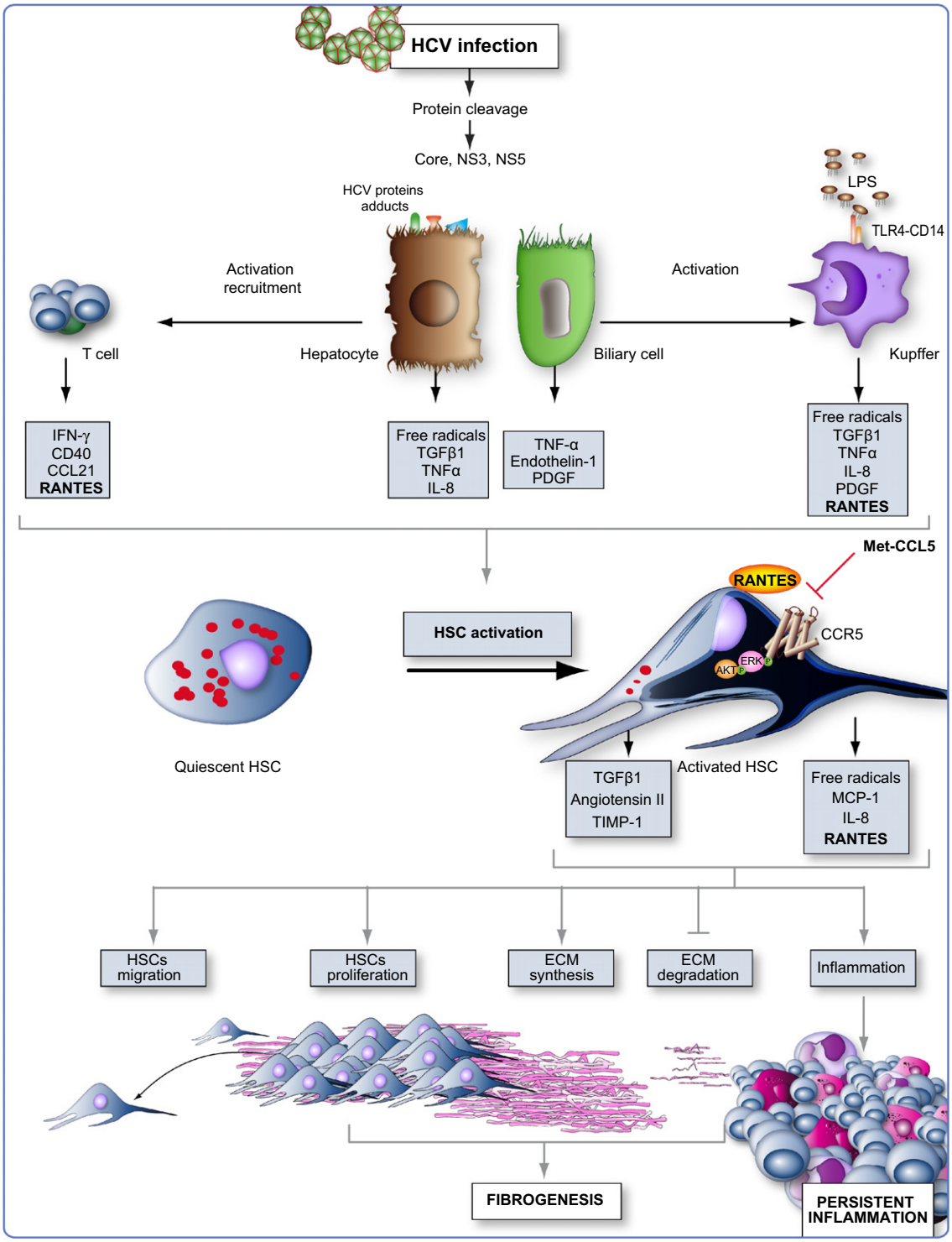

Fig. 1. Implication of RANTES in the pathogenesis of liver fibrosis due to HCV infection. HCV entries hepatocytes and is cleaved into different proteins with biological actions (core, NS3, NS5, etc.). Some of these proteins are expressed in the membrane of hepatocytes together with MHC-I and MHC-II molecules. Moreover, infected hepatocytes secrete inflammatory cytokines. Both actions activate and recruit T-lymphocytes that secrete mediators including RANTES. Neighboring biliary cells and non-parenchymal cells (hepatic stellate cells – HSC and Kupffer cells) become activated and secrete free radicals and fibrogenic and inflammatory mediators. The inflammatory milieu activates resident HSC into myofibroblastic cells. These latter cells express CCR5 and secrete RANTES. Paracrine and autacrine actions of RANTES in HSCs stimulate intracellular signaling pathways leading to increased collagen synthesis, impaired collagen degradation and secretion of further inflammatory mediators. These actions lead to progressive fibrosis and persistent liver inflammation. The use of new RANTES receptor antagonists (e.g. Met-CCL5) could block the pathogenic effects of RANTES and attenuate the progression of liver fibrosis.

Experimental and human evidence indicate that RANTES is implicated in hepatic wound healing response to chronic injury. An initial study demonstrated that RANTES is expressed and secreted by activated HSCs, which are the main collagen-producing cells in the injured liver. In these cells, RANTES induces migration, proliferation, and fibrogenic properties [10]. Moreover, studies in experimental and human liver fibrosis convincingly showed that the CCL5/CCR5 axis is an important system in the hepatic would healing response. This axis is over-expressed in different models of liver fibrosis and in patients with chronic liver diseases such as chronic HCV infection (Fig. 1). Importantly, deletion of CCR5 markedly attenuated liver fibrosis in mice [11]. The underlying mechanisms include modulation of infiltration of T lymphocytes and bone-marrow derived cells and reduced HSCs activation. Finally, recent data suggest that RANTES could be involved in the chemotaxis of progenitor cells during hepatic fibrogenesis and tissue repair [12].

In the paper by Berres *et al.* [6], the authors expanded these previous data by performing a multi-approach study demonstrating that RANTES is a major driver of inflammation and fibrosis in chronic liver injury. First, a genetic analysis indicated that RANTES gene variations influence the degree of liver fibrosis in patients with chronic hepatitis C. The haplotype CCL5_H3 was found more prevalent in patients with advanced fibrosis compared with those with mild fibrosis. Second, RANTES mRNA and protein expression were up-regulated in two experimental models of liver fibrosis as well as in patients with advanced HCV-induced fibrosis. Third, they demonstrated that genetic ablation of RANTES results in attenuated liver fibrosis in mice subjected to two experimental models of liver fibrosis. Absence of RANTES was associated with reduced HSCs activation and immune cell infiltration in the injured liver. By studying bone marrow chimeric mice, they provide evidence that immune cells are the main source of RANTES in liver fibrogenesis, while resident HSCs are probably a target cell type for this chemokine. Finally, the authors tested a recently developed RANTES receptor antagonist (Met-CCL5) *in vitro* and *in vivo*. Met-CCL5 inhibited the proliferation and migration of cultured HSCs as well as chemokine secretion and collagen synthesis. Furthermore, RANTES inhibition attenuated the progression of liver fibrosis in mice treated with CCl_4 and was able to accelerate fibrosis regression after cessation of liver injury.

The study by Berres *et al.* provides convincing pre-clinical evidence that RANTES inhibition is a promising approach to treat chronic liver diseases. However, there are several issues that deserve further attention. The effect of RANTES inhibition on regression of liver fibrosis was only mild and additional studies using different experimental models are needed. Also, the expression of RANTES and its receptors in alcoholic liver disease, which is mainly driven by polymorphonuclear cells, should be explored. Because the authors propose that RANTES is involved in disease progression in chronic hepatitis C, it seems pertinent to explore whether RANTES inhibition modulates HCV cell cycle, replication, and pathogenic effects. For this purpose, *in vitro* replicon systems and transgenic mice are available experimental tools.

Additional studies are also required to better delineate the role of RANTES in liver fibrogenesis and its potential as a target for therapy in humans. Translational studies in different degrees and types of chronic liver diseases should identify the specific cell origin of RANTES. The pathogenic effects of RANTES in mediating hepatocellular injury, endothelial dysfunction, immune disturbances, and collagen synthesis are largely unknown and deserve further investigation. Importantly, future studies should investigate the involvement of CCL5/CCR5 axis in liver regeneration and cancer development. And finally, carefully-designed experimental studies should explore the potential side effects of continuous inhibition in RANTES and/or its receptors in animals with chronic liver injury. In this line, a recent report [13] indicates that lack of CCR5 promotes murine fulminant liver failure by preventing the apoptosis of activated CD1d-restricted NKT cells. This study suggests that prolonged manipulation of chemokine receptors may result in tissue damage instead of resolution of inflammation. Another potential side effect of prolonged RANTES inhibition is inducing immunosuppression. This is particularly important in patients with liver cirrhosis, acute-on-chronic liver disease, and patients with alcoholic liver disease, who are prone to develop severe bacterial infections due to impaired immune defense.

Although chemokines including RANTES are currently considered an appealing family of molecular targets to develop antifibrotic therapies, all these biological and clinical parameters should be carefully considered before testing this type of drugs in patients with chronic liver diseases.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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