IL-17 Signaling Promotes Excessive Alcoholic Liver Disease and Excessive Alcohol Drinking in Mice

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Professor David Brenner, Vice Chancellor for Health Sciences and Distinguished Professor of Medicine at UC San Diego, gave the State of the Art Lecture, entitled “IL-17 Signaling Promotes Excessive Alcoholic Liver Disease and Excessive Alcohol Drinking in Mice.”

In his talk Dr. Brenner wove together separate findings on Alcoholic Liver Disease (ALD) severity, Hepatocellular Cancer (HCC) and Alcohol Abuse Disorder, with the connecting thread being signaling via the cytokine IL-17.

While IL-17 signaling has been implicated in the pathogenesis of NASH- and ALD-induced fibrosis HCC since 2011, the contributions of immune system cells and the hepatocytes themselves to this IL-17 involvement have been unclear. The very recent work of Dr. Brenner described in the latter half of his talk sought to distinguish the contributions of IL-17 signaling in separate cell lineages to liver pathogenesis. Surprisingly, the exacerbation of liver disease by IL-17 signaling was not limited to its actions in immune cells; IL-17 signaling in hepatocytes also had a major impact.

Again and again in his talk, Dr. Brenner relied on multiple mouse models of ALD and HCC to confirm findings, and also used both genetic knockout approaches and pharmacological inhibition to show the role of IL-17 signaling in liver disease. The latter approach also holds out hope for clinical application to liver disease, since IL-17 monoclonal antibodies are already being used to treat autoimmune diseases like multiple sclerosis, rheumatoid arthritis and psoriasis in patients.

What is IL-17, how does it work, and what led to its study in liver disease?

IL-17 is actually a family of cytokines, but generally refers to its most common form, the homodimer of IL-17A. This cytokine is released by a subset of pro-inflammatory T helper cells known as TH17 cells. As with IL-17 itself, there is also a family of receptors, but all receptors have at least one IL-17 Receptor-A chain so that knocking out this one gene, IL-17ra, blocks all IL-17 signaling.

IL-17 signaling activates a large variety of pathways, such as the MAP kinases, NF-κB and STAT3, but its effects are generally weak, and it tends to work in synergy with other inflammatory pathways. One function is to make RNA-binding proteins that stabilize other mRNAs, such as that of TNF-α. Thus it works in numerous ways, but is not directly responsible for much inflammation.
The first hint that it might be relevant to alcoholic liver disease came from study of clinical samples. The level of IL-17 Receptor-A expression was observed to rise with the stage of fibrosis, suggesting the IL-17 signaling activity rose as the disease progressed.

The Tsukamoto-French intragastric alcohol feeding model

The initial experiments Prof. Brenner and Prof. Kisseleva introduced used the Tsukamoto-French intragastric alcohol feeding model in which a intragastric feeding tube is implanted that allows liquid food and alcohol to be pumped directly into a mouse’s stomach, with the advantage that calories and alcohol can both be delivered in precise, known amounts. Gavin Arteel in Ron Thurman’s lab adapted this technique from rats to the use in smaller mice, which was critical, allowing this model to be used in a wide variety of knockout mouse lines to explore what genes are important in ALD. This model’s main shortcoming is that the mice do not go on to develop HCC over their short life spans, so that other mouse models are needed to study that. But the mice do develop hepatic steatosis, inflammation and fibrosis.

IL-17Ra Knockout protects against inflammation, steatosis and fibrosis

By every microscopy technique and indicator used, the livers of IL-17Ra knockout mice look healthier than their wild-type counterparts when subjected to a high-fat + ETOH regimen. This is not to say that there was no liver damage, but rather that it was less in the knockout mice. Serum ALT levels were lower, and the cells were less steatotic and fibrotic. There was less desmin, indicating fewer stellate cells, and there was less F4-80, reflecting less macrophage infiltration. Lipid peroxidation was dramatically less, as seen in lower levels of 4-hydroxynonenal (4-HNE). Expression of virtually all the fibrotic genes was lower. Nearly all inflammatory cytokines were also less, except for IL-10, which is an anti-inflammatory cytokine; IL-10 levels were increased in the IL-17Ra knockout mice.

Inhibiting IL-17 signaling pharmacologically

There are at least three different ways to lower IL-17 signaling pharmacologically. As mentioned above, monoclonal antibodies against IL-17 are in clinical use. Second, there’s an upstream stimulator of TH17 cells called “IL-23.” Blocking IL-23 effectively blocks release of IL-17 upstream. And third, there is a transcription factor, RORγt, that is required for TH17 cells to work and secrete IL-17. Suitable chemical inhibitors are available. So the IL-17 antibody and the RORγt inhibitor were tried, and the results were compared to knockout of the IL-17 receptor.

Pharmacological inhibition of IL-17 also protects the liver

Using the same intragastric alcohol model, both ways of blocking IL-17 attenuated ALD. ALT serum levels were decreased slightly, but blood alcohol levels were confirmed to be the same between the IL-17-inhibited and non-inhibited mice. Steatosis, fibrosis, and inflammation were all decreased. Expression of fibrotic genes and inflammatory genes also decreased. Thus blocking IL-17 pharmacologically by either method gives qualitatively similar results to the genetic manipulation of knocking out the receptor.
Alcohol affects the microbiome, but IL-17 does not

Since microbiome dysbiosis is known to contribute to alcoholic liver disease, it was examined to see whether genetic or pharmacological blocking of IL-17 signaling affected the microbiome itself. The answer was “no.” Alcohol intake affected the microbiome regardless of IL-17 genetic or pharmacological status. Thus blocking IL-17 signaling protects the liver directly without affecting the microbiome.

The DEN carcinogen mouse NASH model of HCC development

As mentioned before, to see HCC develop over the short mouse lifespan, a different model is needed. The best-known model for this is to expose young mice to the carcinogen diethylnitrosamine (DEN), and then subject them to a high-fat diet. Prof. Kisseleva and others showed that adding alcohol to the high-fat diet would accelerate the development of HCC in this model. After 18 weeks of the high-fat diet and alcohol, there is a dramatic induction of HCC tumors and IL-17 levels also rise.

IL-17ra knockout attenuates alcohol-induced HCC

Wild-type and IL-17ra knockout mice were treated with DEN followed by the high-fat diet and ethanol. Alcohol levels were exactly the same, but the tumor burden and liver size were much less in the knockout mice. Known mediators of HCC, such as TNFα and the NADPH oxidases, were also less in the knockout mice. Markers that reflect HCC malignancy such as a-fetoprotein (AFP) and yes-associated protein (YAP) levels were also down in the tumor tissues of IL-17ra knockout mice. In fact, it is possible to passively transmit the HCC tumor cells to untreated mice to monitor in vivo malignancy, and the HCC that formed in the IL-17ra knockout mice was less efficient at transmission into naive recipient wild-type mice.

Two different mouse models of HCC showed a similar response to IL-17ra knockout

Since HCC arises in human ALD without exposure to a separate carcinogen, it was important to use an additional mouse model. In the model developed by Michael Karin, the serine protease Uroki-
tional process has its own characteristic profile of mutations, which is sometimes called its “Alexandrov signatures” after Ludmil Alexandrov, the UCSD scientist who developed this approach to mutation analysis.

First of all, the Alexandrov signatures looked very similar in mouse HCC and human HCC, so the mouse models appear to accurately reflect what happens in human HCC. However, it turned out that IL-17 signaling had no effect on the number of mutations or the mutational signatures of HCC tumors in the mice. Thus the most trivial potential explanation for IL-17’s effects does not seem true. But what was dramatically affected were the gene expression profiles in the presence and absence of IL-17 signaling, and this was true for both tumor and non-tumor tissues.

**IL17ra tissue-specific knockouts in macrophage, stellate cells, and hepatocytes**

Nearly every cell in the body has IL-17 receptors, but in which cells are the responses to IL-17 most important in the development of ALD and HCC? To answer this question, tissue-specific IL-17ra knockouts in macrophage, stellate cells or hepatocytes were constructed and studied\(^1\). Previous studies had not found any response of normal hepatocytes to IL-17, but those studies had not looked at steatotic hepatocytes. As it turned out, we found that knocking out IL-17ra in either macrophage or hepatocytes gave protection against fibrosis, as well as other protective effects that differed depending on the knockout tissue. In macrophage, loss of IL-17ra also reduced inflammation as suspected, because macrophage are responsible for releasing various inflammatory and fibrotic cytokines.

In contrast, the protective effects of IL-17ra knockout in hepatocytes were surprising, given the lack of response of normal hepatocytes exposed to IL-17. Not only was fibrosis reduced, but there was also less steatosis and less HCC than in wild-type mice, despite little or no change in inflammation. These results suggested that there is something special about steatotic hepatocytes. While HCC could still be induced, the tumors were far less malignant as measured by a range of parameters compared to wild-type. AFP and YAP were much less, and gene expression profiling showed less p53, less cytokines and less cytokine receptors being expressed.

**Cholesterol levels down in HCC from IL-17ra\(^{ΔHep}\) mice**

The cholesterol levels, and the levels of two alternative immediate precursors, desmosterol and cholestanol, were down significantly in HCC from the hepatocyte-specific knockout mice compared to the wild-type. The enzyme that makes both of these precursors, 7-dehydrocholesterol reductase (DHCR7), was also present at only 50% of the level seen in wild-type. The precursor of cholestanol, 7-dehydrocholesterol, also serves as a precursor of vitamin D. Consistent with a bottleneck in the conversion of this precursor to cholestanol, an increase in vitamin D was observed.

So why might a decrease in cholesterol synthesis be important in cancer development? Without sufficient cholesterol, the cells can’t make membrane, they can’t proliferate, and they can’t metastasize. The extra vitamin D present is also thought to exert anti-cancer effects.

**IL-17 signaling enables an alternative pathway for inducing cholesterol synthesis**

Michael Karin, also at UCSD, showed that activating TNF-α activates an alternative pathway for cholesterol synthesis that involves caspase-2\(^2\). In fact, the expression of the TNF-α receptor itself on the cell surface is downregulated in IL-17ra knockout mice. The difference was not at the mRNA level, because mRNA levels were exactly the same. Thus something was removing it from the cell surface, and it was possible to see it disappear from the cell surface and appear in the medium. This might happen either through receptor shedding via proteolytic cleavage of the TNF receptor or through exocytosis. These two possibilities can be distinguished using inhibitors of membrane proteases and inhibitors of exocytosis. It turned out that the protease inhibitors had no effect, but the exocytosis inhibitor kept the TNF receptor on the membrane. Thus, there is something about IL-17 signaling that is keeping the TNF receptor on the plasma membrane.
Further studies enabled the Karin group to propose a model in their Cell paper in which TNF activation of the TNF receptor activates caspase-2, caspase-2 in turn proteolytically activates site 1 protease (S1P), and S1P provides an alternative pathway to activate the SREBP transcription factors 1 and 2 that control the synthesis of fatty acid and cholesterol biosynthetic enzymes. The DHCR7 enzyme mentioned above is one of these.

**Further evidence that DHCR7 enzyme levels are key in HCC susceptibility**

If the roughly 50% decrease in DHCR7 expression level observed in the treated IL-17 knockout mice is key to determining cholesterol levels in these hepatocytes, could this decrease be accomplished in another way? While a homozygous knockout of the DHCR7 is embryonic lethal, they were able to make viable mice that were heterozygous for the knockout. In a coup de grâce experiment, they were able to show that these DHCR7 heterozygous knockout mice were remarkably protected against HCC, very similar to the IL-17raΔHep mice. Thus one of the things that IL-17 signaling is doing, maybe the main thing with respect to HCC development, is keeping the TNF receptor on the membrane, enabling the alternative pathway for induction of cholesterol synthesis.

Professor Brenner proceeded to talk about his group’s unpublished work on the role of IL17-signal in alcohol dependence. They were able to demonstrate that pharmacological blockade of IL-17 signaling effectively reduced voluntary alcohol drinking in alcohol-dependent mice, and blocked alcohol-induced hepatocellular and neurological damage. We all eagerly await the formal publication of the new ground-breaking work.

**References**

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