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IL-27 and viral hepatitis: smarter than the average cytokine

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The last few years have seen major advances in the treatment of hepatitis C. Yet despite the use of ribavirin and peg-IFN-alpha therapy more than half of treated patients are unable to develop a sustained viral clearance (SVC), essential for the avoidance of the long term complications of chronic viral infection (1).

Complicating the use of cytokine therapies is the role of the immune system both as a means to clear virus, and inducer of inflammatory liver damage. Cytokines such as IL-10 that limit the action of the immune system, may in the short term relieve liver damage, but at the cost of elevating viral titers and in the long term potentially increase the risk of cirrhosis and hepatocellular carcinoma (2). The perfect cytokine would be one that stimulated a potent antiviral response in the immune system but also had anti-inflammatory properties. Does such a cytokine exist?

T lymphocytes form the backbone of the adaptive immune response to viral infections, both animal models and patient studies have shown the link between a lack of anti-HCV T helper (Th) cell responses and viral persistence (3). Until recently, naive Th cells, when activated were thought to mature into one of two cell fates. These were defined by the cytokines secreted and the unique transcription factors expressed by each lineage. IFN-gamma secreting Th1 cells are thought to be key to combating viral infections. In contrast, interleukin (IL-) 4 and IL-13 secreting Th2 cells protect us from worm infections. The fate of the target T cell is determined by its exposure to cytokines secreted by antigen presenting cells such as dendritic cells at the time of T cell activation (Figure 1). The action of these cytokines is mediated by the phosphorylation and activation of the STAT family of signaling proteins within the target T cell. Phosphorylation of STAT4 and subsequently STAT1 by IL-12 and the interferons (IFN's) mediates Th1 development, whereas phosphorylation of STAT6 by IL-4 induces Th2 development (4). Agents able to shift a Th2 response to a Th1 response would be expected to be beneficial in combating viral infections, a good example of this is peg-IFN-alpha, which is a potent inducer of Th1 cells in humans.

This simple picture has been complicated by the discovery of two further contrasting lineages of cells. The IL-17- and IL-22- secreting pro-inflammatory Th17 cell is implicated in combating extra cellular bacterial and fungal infections and has been linked to a number of mouse models of auto-immune disease (5). In contrast, the anti inflammatory induced T regulatory cell (iTreg) characterized by the expression of the transcription factor FoxP3, inhibits proliferation and cytokine production by other T cells (5). Despite their opposite roles, in mice, Th17 and iTreg cells are both induced by activation in the presence of the cytokine transforming growth factor beta (TGF-beta).

The combination of TGF-beta together with the STAT3 activating cytokine IL-6 producing a Th17 cell (6) where as the combination of TGF-beta and STAT5 activating cytokine IL-2 induces iTreg cell differentiation (7). The key regulating cytokines in humans is not fully

understood although many recent papers suggest similarities to that seen in mice (8). The roles of these two novel lineages in liver disease have yet to be determined, although elevated numbers of Treg cells are associated with HCV viral persistence and antibody depletion of these cells is associated with an *in vitro* enhancement of functional HCV-specific CD8⁺ T cell responses (9). The Th17 cytokine, IL-22, induces STAT3 phosphorylation in its target cell and has been associated with protection against organ damage in the ConA induced mouse model of hepatitis (10).

IL-27 is a member of the IL-6 family of cytokines that include IL-12 and IL-23, like IL-12 it is secreted by activated antigen presenting cells and was originally reported to induce the formation of anti viral Th1 cells (11). Mice lacking the IL-27 receptor have a potent Th2 response and are able to rapidly clear the nematode parasite *T. muris* (12). Similarly IL-27 has been shown to inhibit the differentiation into Th17 cells and iTreg cells *in vitro* by the induction of STAT1 (13). However, unlike IL-12, IL-27 has potent anti-inflammatory properties and there is much evidence that although IL-27 can induce Th1 development *in vitro*, it can act to constrain the inflammatory effect of Th1 cells *in vivo*. In the case of toxoplasma infection, where a Th1 response is critical, mice that lack the IL-12 receptor die from parasite escape, whereas animals that lack the IL-27 receptor die from an excessive inflammatory reaction in part mediated both by Th1 and Th17 cells (13). How IL-27 can both inhibit iTreg formation, induce Th1 cells *in vitro* and yet inhibit inflammation *in vivo* is not fully understood, although there is evidence that IL-27 is a potent inhibitor of T cell proliferation, possibly via inhibition of IL-2. Furthermore, recent research has shown that IL-27 is able to induce T cell secretion of the anti inflammatory cytokine IL-10 (14).

In the current issue of Hepatology by Bender *et al* (Hepatology current issue), the authors look at the actions of IL-27, not on T cells but on hepatocytes. They show that, like in T cells, IL-27 is a weak inducer of STAT3 phosphorylation in hepatocytes and a potent inducer of STAT1, a property it shares with the anti viral interferons. IL-27 is able to induce many anti viral STAT1 dependent genes and the authors demonstrate that *in vitro* IL-27 has a similar action to IFN-alpha in protecting hepatocytes from viral infection. A few caveats remain; IL-27 has been shown to induce IL-10 expression (14), a cytokine linked both to elevated viral titers and an increase in the incidence of hepatocellular carcinoma (2). Conversely, IL-27 inhibits expression of FoxP3, the critical transcription factor for T regulatory cells that have been implicated in sustaining viral persistence (9). IL-10 acts on its target cell by activation of STAT3, implicated in many tumors. As Bender *et al* show in the current issue, IL-27 activates STAT3 in hepatocytes, but to a degree insufficient to activate any STAT3 candidate genes. Nevertheless it remains to be seen whether therapy with IL-27 will be safe as well as efficacious in the treatment of viral hepatitis.

How could these results on IL-27 be taken forward towards therapy for viral hepatitis? First, it would be important to carefully evaluate the impact of this cytokine on viral replication *in vitro*, in particular using the novel systems available for HCV (15). Although the authors show impact on fowl plague virus (FPV), the situation is quite different with persistent infections and with highly adapted human pathogens where it is already known that innate signaling pathways may be specifically disrupted (16). Second, some evaluation of the expression patterns of IL-27 in chronic infection and during conventional therapy would be extremely valuable. There is currently only limited analysis of the cytokine secretion within the liver and its relation to disease outcome. Clearly, this is a complex and technically challenging area. However, it has already been shown that failure of response to conventional IFN-alpha treatment is associated with upregulation of IFN-responsive genes (17), so the extent to which IL-27 signals are up-or downregulated in disease is critical. Treatment of APCs with IFN-alpha has been shown to upregulate IL27 *in vitro* so it is possible that IL-27 activation may come as part of the package with interferon treatment (18). It is worth noting that ribavirin has

pronounced suppressive effects on different cytokines in such systems, and in combination with IFN- α downregulates IL-10 in favour of IL-12, an effect that could have an impact *in vivo* (19).

If we assume that IL-27 continues to show potential as an antiviral cytokine, there still remains a large amount of work to do to understand its possible use *in vivo*. Bender et al show interesting synergy between IL-27 and IFN- α *in vitro*, so further combination might be an attractive approach, in a similar manner to that being taken in the current trials with new oral antiviral drugs. However, the side-effect burden of current treatments is already high and the interactions complex, so simple monotherapy studies with an obvious virologic readout would likely be required to gauge the likely impact of this cytokine. As with Type I interferons, the actual clinical impact is likely to be highly variable between patients, as well as dependent on treatment dose and duration.

Overall, the addition of IL-27 to the already complex array of cytokines with the capacity to modify hepatocyte biology and intrahepatic immunity is an important step in broadening our view of the pathogenesis of viral hepatitis, and its potential therapy. The unusual combination of effects on different cell types and an agent that has both anti-inflammatory and anti viral properties, potentially makes IL-27 “smarter than the average cytokine” and clearly worthy of further study.

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Figure 1.

Actions of IL-27 within the adaptive immune system and liver. IL-27 inhibits Th2, Treg and Th17 lineages and promotes the secretion of IL-10 by Th cells. Dashed arrows represent uncertain or weak effects.

The actions of IL-27 are not universally complementary: Inhibition of Th2 and Treg cells would be expected to aid IL-27 direct ability to inhibit viral replication but this is opposed by IL-27's ability to induce IL-10 secretion.

The role of cytotoxic (CD8+) T cells in this setting is not shown for simplicity.