IFN-γ Inhibition of TGF-β1 Regulates Liver Fibrosis in Young Mice

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ABSTRACT

Introduction: Liver fibrosis is a common response to chronic liver injury. Activation of TGF-β1 is believed to have a critical role. Roles for inhibitory signals such as IFN-γ or Smad7 have not been addressed in children. We have developed a model of hepatitis that is more severe in young 3 week old BALB/c mice in which these mice demonstrate an increased prevalence of fibrosis when compared to controls. Hepatitis and fibrosis are induced following immunizations with liver proteins covalently altered by a model trifluoroacetic acid hapten (TFA-S100). We hypothesize that the pathogenesis of liver fibrosis that develops in young mice following immunizations with TFA-S100 occurs via IFN-γ regulation of TGF-β1.

Methods: 3 week old female BALB/c mice (10 mice per group) were immunized on days 0 and 7 with TFA-S100 emulsified in Complete Freund’s Adjuvant (CFA). Mice also received 500 ng pertussis tox in intramuscularly on day 0. Mice immunized with CFA alone were used as controls. Twenty-one days later the mice were euthanized and their liver and spleen tissues were analyzed. TGF-β1 and IFN-γ levels were measured in liver and spleen supernatants by ELISA (BD, San Diego). Levels were converted to pg/mg to facilitate comparisons between groups. Inflammation/injury and fibrosis were scored as previously described. Statistical analysis was performed using Mann-Whitney U test with P value = 0.05 as statistically significant. Liver homogenates were immunohistochemically analyzed for Smad7 by Western Blot. Tissue fibrosis was assessed by using a Thiohine stain.

Results: 3 week old mice had significantly more inflammation/injury (2.5±0.3) when compared to CFA control (1.1±0.2) (p<0.05). These mice also displayed more fibrosis by histology (5±1) (highest, p<0.05). TGF-β1 levels were elevated in TFA-S100 immunized mice, however the levels were not statistically different from controls. IFN-γ levels were significantly reduced in TFA immunized mice when compared to controls (p<0.05), and corresponding Smad7 protein levels were also markedly lower.

Conclusions: Our findings support the notion that the mechanism of liver fibrosis that develops following immunization with our hapten occurs by IFN-γ regulation of TGF-β1 pathway via Smad7. These studies show a critical role for IFN-γ and suggest that the first time key role for Smad7 in this process in young mice. In susceptible persons, TGF-β1 activation leads to the formation of fibrosis as well as inhibition of IFN-γ. Decreased activation of IFN-γ promotes more fibrosis through reduced Smad7. Even though fibrosis is a key process in healing, current evidence suggests that this healing process, when unchecked, can result in cirrhosis. Hence, further studies are needed to elucidate additional critical pathways that may uncover therapeutic options for children with fibrosis.

METHODS

Drug-induced hepatitis accounts for approximately 50% of acute liver failure in the United States. While the majority of these cases include toxic hepatitis, 13% of these cases are not due to either toxic or idiopathic. In the triply hepatotoxic model, we hypothesize that: 1) metabolic idiopathic hepatitis and 2) immune-mediated idiopathic hepatitis, in which drug metabolism produces reactive drug hapten that alter native proteins, which the body then recognizes as foreign. Children routinely receive many of the problems described associated with the immune-mediated, idiopathic hepatitis.

Liver fibrosis is a response to chronic liver injury. Many mechanisms are believed to have a role in its pathogenesis. Activation of TGF-β1 is believed to have a critical role, while roles for inhibitory signals such as IFN-γ or Smad7 have not been addressed in children. Studies demonstrating key elements of this pathway have been hampered by the lack of a reliable model that recapitulates inflammation and fibrosis seen in liver biopsy without altering mouse survival. We have developed a model of hepatitis that is more severe in 3 week old BALB/c mice in which the mice demonstrate an increased prevalence of fibrosis when compared to controls. Hepatitis and fibrosis are induced following immunizations with liver proteins covalently altered by a model trifluoroacetic acid (TFA) hapten (TFA-S100). We hypothesize that the pathogenesis of liver fibrosis that develops in young mice following immunizations with TFA-S100 occurs via IFN-γ regulation of TGF-β1.

RESULTS

Figure 1. 3 week old female BALB/c mice (10 mice per group) were immunized with CFA±100 μg TFA-S100 on days 0 and 7 (above). Twenty-one days later the mice were euthanized and their liver and spleen tissues were analyzed. 2) A schematic of the sandwich ELISA is demonstrated. (left)

Figure 2. Significantly more inflammation/injury (2.5±0.3) in TFA-S100/CFA group compared to CFA control (1.1±0.2) (p<0.05). Figure 3. Hepatic Fibrosis in 3 week old mice following TFA-S100/CFA immunizations with Hematoxylin and Eosin Staining. Figure 4. Reduced levels of IFN-γ in TFA-immunized mice compared to CFA control (p<0.05). Figure 5. Differences in liver TGF-β1 levels between control CFA and experimental TFA-S100/CFA groups approached significance. Figure 6. Reduced levels of Smad7 in TFA-immunized mice compared to CFA controls.

CONCLUSIONS

Our findings support the notion that liver fibrosis that develops in our model occurs by inhibiting IFN-γ regulation of TGF-β1 producing both that subsequently travel to the liver. We also suggest that IFN-γ regulation may occur via Smad7. These studies show a critical role for IFN-γ and suggest for the first time key role for Smad7 in this process in young mice. Current studies suggest that fibrosis may be a healing process, but when unchecked, may result in cirrhosis. Hence, further studies are needed to elucidate additional critical pathways that may uncover therapeutic options for children with fibrosis.

REFERENCES