pISSN 2287-2728
eISSN 2287-285X

CMH

https://doi.org/10.3350/cmh.2020.0040
Clinical and Molecular Hepatology 2020;26:236-237

Letter to the Editor

Does limited expression of toll-like receptor 9 actually contribute to T cell activation and liver damage in non-alcoholic steatohepatitis?

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Dear Editor,

I recently read an interesting article by Alejandra et al.[1] which showed the relationship between toll-like receptor 9 (TLR9), cluster of differentiation 69 (CD69) expression, and interferon-γ expression related to liver injury in patients with nonalcoholic fatty liver disease. Non-alcoholic fatty liver disease involves a comprehensive process from simple fatty liver to nonalcoholic steatohepatitis (NASH), which includes hepatocellular damage (hepatocyte ballooning) due to infiltration of inflammatory cells as well as fat accumulation, which thereby progresses to NASH-associated fibrosis and NASH-associated cirrhosis.[2-4] The authors investigated the expressions of TLR9 and CD69, as well as the frequency of interferon-γ positive cells after anti-CD3 and TLR9 ligand (*CpG* oligodeoxynucleotid) stimulation, which were examined in peripheral blood cells isolated from normal control, simple steatosis (SS) patients, and NASH patients. In comparing the CD4 and CD8 T cells of SS patients with the cells of normal controls and NASH patients, a number of different aspects were found: 1) TLR9 expression on T cells, 2) CD69 expression by anti-CD3 and TLR9 ligand stimulation, and 3) interferon-γ positive cells. NASH patients had a significantly higher percentage of interferon-γ positive circulating cells compared to the others. In fact, the decreased expression of TLR9 on T cells and reduced interferon-γ expressing T cells in SS patients may play a protective role against liver damage. However, there are several things to consider regarding this hypothesis. I have briefly summarized the results, as shown in the table below, to enhance the readers' understanding (Table 1).[1]

First, the authors analyzed TLR9 expressions in both intrahepatic and peripheral CD4 T and CD8 T cells. However, only peripheral blood mononuclear cells were used to examine the expressions of CD69 or interferon-γ. Since hepatocellular damage in NASH patients could actually be caused by intrahepatic immune cells, it remains somewhat questionable whether peripheral blood analysis can really reflect intrahepatic immune cells. Also, intrahepatic immune cells showed significant differences in phenotype and composition of immune cells located in other organs, such as the spleen lymph nodes, and etc.[5]

Second, it is not clear whether these processes were caused by a change in TLR9 ligand responsiveness for T cell activation or by other factors, such as a difference in the influx of TLR9 ligands into the liver between SS and NASH patients. The authors suggested that in patients with SS, T cell activation and induction of interferon-γ positive cells were blunted due to low TLR9 stimulation on CD8 T cells, resulting in less liver damage compared to

Abbreviations:

CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CD69, cluster of differentiation 69; NASH, nonalcoholic steatohepatitis; SS, simple steatosis; TLR9, toll-like receptor 9

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Editor: Seung Up Kim, Yonsei University College of Medicine, Korea

Received : Feb. 27, 2020 / **Revised :** Mar. 4, 2020 / **Accepted :** Mar. 5, 2020

Table 1. TLR9, CD69, and interferon-γ expression on T cell for control, SS, and NASH patients

	TLR ligand influx increase/ decrease	TLR9 expression level	CD69 expression after anti-CD3, TLR9 ligand stimulation	Interferon-γ expression levels by anti-CD3 and TLR9 ligand stimulation	Interferon-γ expression level
Control					
Intrahepatic	–				
Peripheral	–				
SS					
Intrahepatic	–	CD4 T ↓ CD8 T →	–	–	–
Peripheral	–	CD4 T ↓ CD8 T ↓	CD4 T → CD8 T ↓	CD4 T → CD8 T ↓	CD4 T → CD8 T →
NASH					
Intrahepatic	–	CD4 T → CD8 T →	–	–	–
Peripheral	–	CD4 T → CD8 T →	CD4 T → CD8 T → (CD8 T ↑ compared to SS)	CD4 T → CD8 T → (CD8 T ↑ compared to SS)	CD4 T ↑ CD8 T ↑

TLR, toll-like receptor; CD, cluster of differentiation; SS, simple steatosis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; NASH, nonalcoholic steatohepatitis.

NASH patients. It seems that T cells of each group may appear to show a difference in activation by TLR9 ligand stimulation under controlled laboratory conditions. However, the results for comparing the actual interferon-γ positive circulating T cells isolated from patients did not show any significant differences in SS patients compared to NASH patients. This suggests that other factors could also take part in leading to liver damage under *in vivo* conditions. Indeed, SS progresses to NASH via a complex process involving many factors, such as genetic susceptibility, various environmental effects, and immunological factors.[4,6] For example, when TLR9 ligand enters the liver, hepatic stellate cells and Kupffer cells may react with the ligand and produce pro-inflammatory cytokine, which induces secondary activation of CD4 and CD8 T cells.[7] Therefore, it would be more clear if additional supporting results were available, such as a ratio comparison of intrahepatic interferon-γ positive CD4 and CD8 T cells in normal control, SS model, and NASH model isolated from mice lacking TLR9 in the myeloid cells (TLR9 f/f X Lysozyme M-Cre mouse).[7]

Objective evidence should be provided on whether there is actually a difference in TLR9 ligand influx into the liver between NASH and SS patients. Further experiments on the aforementioned points would help solve the questions raised in this study.

Conflicts of Interest

The author has no conflicts to disclose.

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