Review

The Pathogenesis of Acute on Chronic Hepatitis B Liver Failure

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Acute-on-chronic liver failure is a characteristic clinical liver syndrome, which should be differentiated from acute liver failure, acute decompensated liver cirrhosis and chronic liver failure. The pathogenesis of ACLF is not fully understood yet. Viral factors and immune injury have been reported to be the two major pathogenesis. This paper reviewed the researches on the pathogenesis of acute on chronic hepatitis B liver failure in recent years, to provide theoretical basis for prompt and accurate diagnosis and treatment of this syndrome. This would benefit for the prognosis and raise the survival rate of patients.

Key words: Pathogenesis; Acute on chronic hepatitis B liver failure; Cytokine; Immune injury

Acute on chronic liver failure (ACLF) is a characteristic clinical syndrome with poor prognosis. The manifestations of ACLF are based on severe chronic hepatitis. The vast majority of patients with ACLF always suffered from cirrhosis and deteriorated liver function. Acute liver failure occurring under general condition is not a good phenomenon. At present, the worldwide definition and diagnostic criteria of ACLF is still controversial.

The common feature of ACLF is that it can contribute to not only liver failure but also multiple organ failure, especially renal failure. Although the clinical features of liver failure are similar, prognosis are different. The mortality of the patients with ACLF in 28 days and 3 months are 33.9% and 51.2%, respectively. The mortality of acute decompensated liver cirrhosis (ADC) in the 28 days and 3 months are 1.9% and 9.7%, respectively.¹ ACLF occurring on the background of chronic liver disease often has obvious precipitating factors, such as bleeding, infection, poisoning. Therefore, ACLF should be differential diagnosed with identification of chronic liver failure, acute hepatic failure (AHF) and ADC.

In China, 80% ACLF were caused by acute exacerbation of chronic hepatitis B (CHB). The pathogenesis is very complex and has not been fully elucidated. There is no systemic review on the two major pathogenesis including virus factors and

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world.

SPECIFIC T CELL FAILURE

Long-term persistent HBV infection is an important factor leading to ACLF. Immunological factors may be important factors involved in the pathogenesis of ACLF. Antigen-presenting cells especially dendritic cells (DC) can reduce the number and deteriorate the function of T cells. While stellate cells and sinusoidal endothelial cells can present HBV antigen stimulation and the signal is too weak for T cells to tolerate.² The number of regulatory T cell (Treg) increases significantly and is positively associated with HBV DNA load. T regulating cells (Tregs), composition and function of which can be reflected by T cell receptors (TCR), play an important role in maintaining immunological tolerance to self and foreign antigens. The most prevalent T cell receptor β chain variation (TCRBV) in CD4⁺CD25⁺ Tregs in ACLF and CHB patients includes TCRBV11, BV13.1, BV18, BV20. TCRBV20 CDR3 (complement, complementarity determining region 3) modified "TGTGHSPHLH" and TCRBV11 "VYNEQ" CDR3 for diagnosis of ACLF.³ The expression of some co-inhibitory molecules increased on the surface of the T cells, including PD-1 (programmed death-1, or programmed cell death 1), CTLA-4 (cytotoxic T lymphocyte associated antigen-4 and Tim-3 of T cell immunoglobulin domain and mucin 3) and so on. These co-inhibitory molecules, such as PD-1 can significantly inhibit the secretion of IFN- γ , the function of IL-12 and the

activity and proliferation of NK cells. High expression of CTLA-4 inhibitory molecules can increase HBV specific proapoptotic protein Bim (Bcl-2 interference medium), mainly through the induction of CD127 low expression, resulting in HBV specific T cell apoptosis. At the same time, Bim made T cells secrete a small amount of IFN- γ , and not disintegrate into memory T cells.⁴ Tim-3 can reduce the cytokine production.⁵ High expression of multiple co-inhibitory molecules is the key cause to deplete the function of HBV specific T cell. Therefore blocking these co-inhibitory molecules can contribute to significant restoration of HBV specific T cells and removal of the virus.

The study showed that CHB was characterized by functionally impaired virus-specific CD8⁺ T-cell responses, especially increased expression of Bim (a proapoptotic protein). In the body of patients with acute on chronic hepatitis B liver failure (ACHBLF), nonspecific CD8⁺ T cells are dysfunctional, the capacity to produce IL-2 and proliferation of CD8⁺ T cells are significantly decreased, but the function of proinflammatory factor including IFN- α and IFN- γ is not damaged, which causes nonspecific inflammatory injury.

STELLATE CELLS IN THE PATHOGENESIS OF ACLF

The experimental data showed that the HSCs (hepatic stellate cells) also had characteristics of liver stem/progenitor cells (HPCs). Liver biopsy with histochemistry analysis showed that the number of activated HSCs and HPCs was higher in ACLF than in acute hepatitis and other chronic liver disease ($P < 0.001$). Meanwhile it was found that there's a significant correlation between the presence of HSCs and HPCs, which points out the HSCs can initiate liver regeneration and pathobiology of ACLF. Rastogi et al gave a report on liver biopsy of 70 cases of ACLF, which showed significant more α -SMA activated HSCs in ACLF biopsies than in biopsies of patients with acute hepatitis, chronic hepatitis, and normal LDLT donor biopsies.⁶ So it suggested a significant correlation between HSCs and ACLF.

THE GENOTYPE OF HBV AND ACLF

In recent years, a correlation between the HBV genotype and ACLF has been found. It was pointed out in that C53T, A1846T and G1896A are the most

common ACLF gene mutations. Cross sectional study showed that 61% of ACLF patients, 11.1% of chronic hepatitis B (CHB) patients, 31.1% of liver cirrhosis (LC) patients and 33.3% of hepatocellular carcinoma (HCC) patients carried T1864. The percentage of patients with ACLF, CHB, LC, HCC carrying A/G1913 were 42.9%, 2.2%, 17.8% and 11.1%, respectively. In addition, they also found the incidence of T1846 was much higher in patients infected with B genotype (57.1%) than those with C genotype (30.4%). The A/G1913 gene in HBeAg negative patients (28%) was higher than that in HBeAg positive patients (13.2%). Multivariate analysis indicated that T1846 and A/G1913 were independent factors of ACLF, whose gene mutations were closely associated with the pathogenesis of ACLF.⁷

HBV genotype and mutations in patients with ACHBLF are located in the basal core promoter (BCP) and precore (PC) regions. Xiao L et al have done a survey on 322 patients with chronic HBV infection, including 77 cases of ACHBLF, 109 cases of HCC, and 136 cases of CHB.⁸ Among them, there were 61.5% patients with B genotypes, and 38.5% patients with C genotypes. The percentage of patients with ACHBLF with B genotype was significantly higher than that with CHB (92.2% vs. 60.3%, $P < 0.001$), whereas the percentage of patients with ACHBLF with C genotype with HCC was higher than that with CHB. With B genotype A1762T/G1764A, A1846T and G1896A gene mutations, the incidence of ACLF was higher than CHB and the incidence of liver failure was also high.

TH17(CD4⁺T) CELLS AND IL-17 AND ACLF

The Th17 cells associated with liver injury are involved in the pathogenesis of hepatitis B. Th17 cells, whose mechanism is still unknown, can induce immune activation and disease aggravation.⁹ In patients with ACHBLF, Th17 cells and serum IL-17 concentration is significantly higher than that with CHB, chronic asymptomatic HBV carriers and normal control group. Meanwhile it is worth mentioning that the increase degree is associated with immune inflammation. Th17 cell in the peripheral blood of patients with ACLF is also associated with immune inflammation. Associated with INR, MELD score were positively related, while the level of IL-17 decreased gradually with improvement. ACHBLF Treg/Th17 ratio may be related to the prognosis of the

disease and HBV persistent infection.¹⁰

LIPOPOLYSACCHARIDE, TOLL LIKE RECEPTOR AND ACLF

It has proved in animal model that high serum lipopolysaccharide (LPS) and LPS-MD-2 (lipopolysaccharide myeloid differentiation protein-2/TLR4, Toll like receptor 4) complex activated NF- κ B and cytokines can induce hepatocyte necrosis. Patients with ACHBLF has dynamic changes of LPS in different disease stage, and has the highest level in the peak phase. The dynamic changes of LPS were related with the severity of disease and may induce secondary liver injury.¹¹

TLR3 plays a key role in innate immunity and identification of viral pathogens. TLR3 A952T and C1234T are two polymorphisms. Rong et al have done a survey including 452 cases of chronic hepatitis B and 462 cases of healthy control group.¹² The results indicated that subjects carrying C1234T genotype and TT genotype had 1.42-fold and 2.31-fold increased risk of chronic HBV infection compared to those with CC genotype. Further analysis showed the prevalence of C1234T genotype and T allele in CHB patients with ACLF was significantly higher than that in patients without ACLF. These results indicate that TLR3 C1234T distribution is a risk factor for development of chronic HBV infection, especially the CHB-related ACLF.

GLUTATHIONE-S-TRANSFERASE AND ACLF

Glutathione-S-transferase M3 (GSTM3) belongs to GSTs family, which can contribute to oxidative stress-mediating liver damage. The study of GSTM3 gene promoter methylation and oxidative stress in ACHBLF patients showed that GSTM3 promoter methylation rate of ACHBLF is higher than that of CHB (30% vs. 6.7%, $P < 0.05$). Plasma malondialdehyde (MDA) and GST levels in patients with CHB were significantly increased. Plasma MDA and GST levels were much higher in ACHBLF patients than in CHB patients. At the same time, in ACHBLF patients, MDA, MELD scores and mortality rate were much higher in methylated group than those in unmethylated group. Severity about GSTM3 gene promoter methylation and oxidative stress may lead to liver injury and disease of ACHBLF.¹³

Glutathione-S-transferase P1 (GSTP1) gene promoter methylation is closely associated with the liver injury caused by oxidative stress in ACHBLF. Li et al found that 11 of 35 patients with ACLF and 3 of 35 patients with stable hepatitis B displayed GSTP1 promoter methylation, and the difference was significant.¹⁴ The levels of MDA adducts were significantly higher in patients with liver failure compared with those with CHB, and in the patients with liver failure who had promoter methylation the levels were higher than in those who did not. The results suggest that GSTP1 promoter methylation may promote oxidative stress-associated liver damage in ACHBLF, and oxidative stress is associated with the severity of ACHBLF.

COX-2 AND PPAR γ AND ACLF

COX-2 (cyclooxygenase-2) and PPAR γ (peroxisome proliferator activated receptor gamma) express in most nucleus of hepatocytes. In liver cells of patients with ACLF or CHB, especially the former, COX-2 expression increased significantly. PPAR γ also increased significantly in liver cells of patients with ACHBLF. In liver cells of patients with ACLF, COX-2 expression is correlated with the MELD score and PPAR γ is associated with the loading of HBV DNA. Clinical data indicated that in liver cells of patients with ACLF, ALT, TBil, CHOL (low density lipoprotein cholesterol), PT, FIB (fibrinogen) and MELD were much higher than those of chronic liver failure, chronic hepatitis B and normal control group. Hepatic COX-2 expression is a marker to reflect the degrees of inflammation and injury of liver tissue, and PPAR γ expression in liver is due to chronic HBV infection, which may be a protective mechanism against liver injury.¹⁵

IL-21 AND ACLF

Immune mechanism induced by ACHBLF has not been known much. IL-21 is a newly discovered cytokine, involved in autoimmune and inflammatory diseases. Its potential role in ACHBLF is still unknown. As determined by flow cytometry bead array, the serum levels of cytokines and IL-21-secretion of CD4⁺ T cells in peripheral blood mononuclear cells (PBMC) were identified by intracellular cytokine staining. The results showed that in the blood of patients with ACHBLF the

secretion of CD4⁺ T cells-IL-21 increased, and IL-21 level in the serum was the highest in HB-ACLF and positively associated with high MELD score and mortality. In the recovery stage of ACHBLF, serum IL-21 level decreased, so did CD4⁺ T cells.

Recombinant human interleukin-21 (rhIL-21) stimulates the secretion of PBMC- IL-1 β , IL-10, IFN γ and TNF α . Therefore, IL-21 is a causative factor in severe liver disease, which increases in ACHBLF, and severe liver disease.¹⁶

CONCLUSIONS

Overexpression of HBsAg in the liver can cause liver cell degeneration and necrosis, HBV genotype and gene mutation are involved in the pathogenesis of ACHBLF. Endotoxemia and hepatic microcirculation of liver failure occur in the process of depletion of T cells and loss of the function of specific T cell. Regulatory T cells increase is another pathogenesis of ACHBLF. Virus, immune injury and cytokines are interact and reciprocal causation which links perplexing, but detailed mechanism remains unknown. This needs further study. Thorough understanding the pathogenesis of ACLFV would be beneficial for the prognosis of ACLF.

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