Research Article

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Protective effect of Th22 cells and intrahepatic IL-22 in drug induced hepatocellular injury

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Background & Aims: Th22 cells regulate host immunity against pathogenic invasion, including protecting host against chronic hepatitis B; however, the relationship between drug induced liver injury (DILI) and Th22/Th17 cells is still unclear. We investigated the role of Th22 cells in DILI development.

Methods: The frequencies of peripheral Th22/Th17/Th1 cells and intrahepatic IL-22/IL-17 production from DILI, non-DILI liver diseases, and healthy controls were examined. Plasma IL-22/IL-17 and the related cytokines were determined in DILI patients at week 0 (defined as the occurrence of liver injury within 7 days), 4 and 24. Multivariable stepwise logistic regression was applied to explore the associations between various factors and recovery of DILI.

Results: The frequencies of Th22/Th17 cells were significantly higher in DILI onset patients than HC. Significant increase of Th22 cells and the related cytokines levels was observed in DILI with hepatocellular injury type. There was a positive correlation between intrahepatic IL-22 level and liver regeneration. Plasma IL-22 level was higher in DILI patients with improved liver function than unimproved function. Multivariable analysis showed that the odds ratio (OR) of plasma IL-22 at 4 weeks was 1.054 [95% confidence interval (CI), 1.012, 1.124].

Conclusions: Increased peripheral and intrahepatic IL-22-secreting cells are detected in DILI. Th22 and its related cytokines might be hepato-protective, which might provide new perspective for understanding the immunopathogenesis of DILI. Plasma IL-22 might be a reliable indicator to evaluate the prognosis of DILI and provide a novel therapeutic target for DILI treatment.

Keywords: Th22 cells; Th17 cells; IL-22; DILI; Protection.

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Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; AIH, autoimmune hepatitis; CHB, chronic hepatitis B; CK 7, cytokeratin 7; DILI, drug induced liver injury; HAI, histological activity index; IL-22, interleukin-22; IL-17, interleukin-17; NSAIDs, non-steroidal anti-inflammatory drugs; PCNA, proliferating cell nuclear antigen; RUCAM, The Roussel Uclaf Causality Assessment Method; TCM, Traditional Chinese Medicine; ULN, upper limit of normal.

Introduction

Drug induced liver injury (DILI) is the most common cause of acute liver failure [1]. The pathogenesis of DILI usually involves toxic drugs or metabolites that elicit an immune response, or directly affects the physiology of hepatocytes [2]. The mechanism of the immune-mediated drug reaction may involve a hapten-like action, resulting in autoimmunity against hepatocellular components [2,3]. Lymphocytes were detected from drug induced liver failure patients [4], and neutrophil/macrophage mainly infiltrated around the central vein from DILI patients [5]. Moreover, it was observed that pro-inflammatory cytokines/chemokines release contributed to development of liver injury [6].

IL-17-producing $CD4^+$ T cells (Th17) are implicated in the pathogenesis of DILI [7], and elevated IL-17 levels have been observed [8]. Furthermore, increased Th17 cells level is detected in DILI onset [8], suggesting a correlation between the level of IL-17 and the severity of liver inflammation [9].

Th22 cells, secreting IL-22 but not IL-17 and IFN-γ [10], are involved in the pathogenesis of inflammation [11]. Induction of Th22 cells is involved in IL-6 and TNF [12]. IL-22, a key molecule in the modulation of cellular inflammatory responses [11], is secreted mainly by Th22, Th17, and Th1 cells [11,13]. Our previous report suggests that IL-22 is hepto-protective in chronic hepatitis B patients [14], while another study has suggested that IL-22 is pro-inflammatory in chronic liver diseases [15]. The relationship between DILI and Th22/Th17 remains to be explored.

In this study, the frequencies of Th22 cells, levels of plasma and intrahepatic IL-22, and their association with liver regeneration in DILI patients were investigated. The relationship between Th22-related cytokines and disease progression of DILI was explored. Our results show increased peripheral Th22 and hepatic/plasma IL-22 in DILI onset patients, particularly in hepatocellular injury type. Increased plasma IL-22 in early stage may indicate a better prognosis.

Table 1. Clinical characteristics and suspicious drugs used by 42 DILI patients.

Characteristics	Summary cases (%)
Case number	42
Gender (F/M)	32/10
Age (years)	45.05 ± 1.57
HAI scores	6.02 ± 0.52
Clinical classification	
Hepatocellular	23 (55)
Cholestatic	12 (29)
Mixed	7 (17)
Outcome	
Recovery	34 (81)
Chronicity	8 (19)
Suspicious drugs	
TCM	17 (40)
Antibiotics	11 (26)
NSAIDs	5 (12)
Cardiovascular drugs	4 (10)
Hormonal contraceptives	3 (7)
Others	2 (5)

F, female; M, male; HAI, histological activity index; TCM, Traditional Chinese Medicine; NSAIDs, Non-steroidal anti-inflammatory drugs.
Note 1: Of the 17 cases using TCM, 4 cases took *Radixpolygoni multiflori* (He Shou Wu), commonly used as a dietary healthy supplement in China.

Materials and methods

Recruitment of patients

To identify the frequency of different subsets of functional $CD4^+$ T cells, 107 patients were diagnosed and treated consecutively between April 2012 and October 2013, in the Outpatient and Inpatient Clinic, Department of Infectious Diseases, Ruijin Hospital, China. Forty-five DILI patients were enrolled in the study within 7 days of injury onset; 62 cases including non-DILI liver diseases (AIH, n = 12; CHB, n = 30; AHB, n = 20). Drug-, sex-, and age-matched patients without liver disorders (n = 16) were recruited as non-DILI controls, 30 subjects were healthy controls. DILI patients were treated with glycyrrhizin (average 450 mg/day) or ursodeoxycholic acid (10 mg/kg/day) [16,17]. Liver biopsy was not performed in 3/45 DILI patients due to coagulopathy (INR >1.5).

Liver biopsies from DILI (n = 42), non-DILI liver disease (AIH, n = 12; CHB, n = 19) and four healthy liver transplant donors were collected for immunohistochemistry. The clinical presentations and suspicious drugs caused liver injury were shown (Table 1). Traditional Chinese Medicine (TCM) was the leading culprit of liver injury, accounting for 40%, followed by antibiotics (26%), non-steroidal anti-inflammatory drugs (12%), cardiovascular drugs (10%), contraceptives (7%), anti-ulcer drug (2%), and sleeping pill (2%). Hepatocellular injury was the main clinical pattern in the current study (55%). Clinical data of different liver diseases (DILI, AIH, and CHB) are shown in Supplementary Table 1. Kinetic plasma IL-22/IL-17 and the related cytokines were detected in DILI patients at week 0, 4, and 24.

This study was approved by *the Human Ethics Committee of The Institute*, and written informed consents were obtained.

Diagnosis and classification of DILI

The Roussel Uclaf Causality Assessment Method (RUCAM) [18] was used for DILI diagnose. Only cases that had scored as highly probable or possible ($\geqslant$6 points by RUCAM) were included in this study. The DILI was classified into three categories (International Consensus Meeting for Liver Injury) at the onset of liver injury [18]. The described criteria and classification for DILI are outlined in Supplementary Material.

These patients were further sub-divided into above or below 5-fold ULN groups based on ALT levels, because 5× ULN for ALT is a threshold differential of significant liver injury [19].

To study the relationship between Th22-related cytokines and the course of DILI, we divided the patients into two groups based on the situation of patient as follows: 1) Recovery, defined as liver biochemistry values returned to normal persisting for more than 6 months post-drug withdrawal; 2) Chronicity, defined as persistent biochemical abnormality for more than 6 months after drugs withdrawal [20]. All the patients were followed up for a median of 10 months (6–24 months). Plasma collected from acute DILI patients within 7 days of clinical onset on baseline (week 0, n = 42), week 4 (n = 32) and 24 (n = 21) were stored at −80 °C until assay.

Other Materials and methods are shown in Supplementary Material.

Statistical analysis

All statistics were expressed as mean ± SEM of each group. Differences between two groups were determined by unpaired *t* test or the Mann-Whitney U test, among three groups by analysis of variance (ANOVA) or the Kruskal-Wallis H nonparametric test. Chi-square or Fisher's exact test was employed to compare nominal variables. Spearman correlation was used for correlation analysis. All tests were performed by GraphPad Prism 5.0. p value <0.05 was considered statistically significant. A multivariate stepwise logistic regression was applied to fit a model that identifies independent predictors of recovery. Age, gender, classification of liver injury (hepatocellular, cholestatic, or mixed), degree of liver injury (ALT $\geqslant$5× ULN or ALT <5× ULN), categories of drugs, HAI scores, and cytokines variables (IL-22, IL-6, TNF, IL-17, IL-23, and IFN-γ, at week 0, 4, or 24) were analysed as candidate factors for the outcome. A significant level of 0.05 is required to allow a variable into, and subsequently stay in the model. Multivariate analysis was performed with SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

The frequencies of Th22 cells and the level of Th22-related cytokines in DILI patients with hepatocellular injury were significantly increased

The frequencies of Th1 ($CD4^+IFN\gamma^+IL17A^-IL22^-$), Th17 ($CD4^+IFN\gamma^-IL17A^+IL22^-$), $CD4^+IL22^+$ T cells and Th22 ($CD4^+IFN\gamma^-IL17A^-IL22^+$) cells from the DILI and HC are displayed in dot-plots (Fig. 1A). Th22 cells were 2, 3, 2 or 2-fold higher in DILI, AIH, CHB or AHB groups than HC, respectively (Fig. 1B, p <0.05). Th17 cells were 3, 3, 2 or 2-fold increased in DILI, AIH, CHB or AHB groups compared with HC, respectively (Fig. 1B, p <0.05). In contrast Th1 cells were significantly lower in DILI, AIH, or CHB by >30% compared with HC (Supplementary Fig. 1A, all p <0.05). Circulating Th22 and Th17 cells were significantly higher in the hepatocellular-injured than cholestatic-injured DILI patients (Fig. 1C, all p <0.05). The percentages of Th22/Th17 cells were significantly higher in hepatocellular-injured than that in drug-, sex-, and age-matched patients without liver injury (Fig. 1C, p <0.0001, p <0.001, respectively). However, there was no significant difference of Th1 cells among the various groups determined (Supplementary Fig. 1B). Furthermore, there was a significant correlation between Th22 and Th17 cells from DILI (Fig. 1D, p <0.0001). Additional correlations were also observed between Th22 and $CD4^+IL\text{-}22^+$ cells (Fig. 1D, p <0.0001), as well as between Th17 cells and $CD4^+IL\text{-}22^+$ cells in DILI (Supplementary Fig. 2A, p <0.0001). However there was no correlation between Th22 cells and Th1 cells (Supplementary Fig. 2B, p = 0.2160), as well as between $CD4^+IL\text{-}22^+$ cells and Th1 cells in DILI (Supplementary Fig. 2C, p = 0.2123). There were significant correlations between the frequencies of Th22/Th17 cell and plasma ALT levels in these DILI subjects (Fig. 1E, p = 0.0010; p <0.0001, respectively), except for Th1 cells (data not shown).