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ORIGINAL ARTICLE

Fumarate hydratase-specific T cell response in Chinese patients with autoimmune hepatitis

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KEYWORDS
Autoimmune hepatitis;
Fumarate hydratase;
T cell response;
Cytokines

Summary
Purpose: Fumarate hydratase (FH) is expressed in the serum of patients with autoimmune hepatitis (AIH). The specific involvement of FH-specific T cell response is currently unknown. The aim of the study was to assess the frequency and clinical significance of FH-specific T cell response in AIH.
Methods: This was a prospective study of 42 consecutive patients admitted to the clinical study center of autoimmune liver disease of our Hospital, Capital Medical University (China) between January 2011 and December 2014. PBMCs were collected and the FH-specific T cell response was detected by Elispot. Cytokines and antibody responses were assessed.
Results: Among the 42 AIH patients, 57.1% showed a positive response to FH peptides. The difference in FH-specific T cell response frequency among AIH patients and control groups was significant ($P < 0.001$). The FH peptides induced the secretion of CD4+ and CD8+ T cells. The FH-specific T cell response in patients with active disease was stronger than in those with remission ($P = 0.0283$). FH-specific T cell response in patients with active disease showed a positive association with ALT ($r = 0.4712$, $P = 0.0098$) and AST ($r = 0.3924$, $P = 0.0352$) levels. The magnitude of the FH-specific T cell response correlated with the HAI score ($r = 0.7290$, $P = 0.0047$) and anti-FH titer ($r = 0.6457$, $P = 0.0093$).
Conclusion: FH-specific T cell response may be detected in the blood of patients with AIH and seems to be associated with AIH disease progression. FH-specific T cell response could be a pathogenic cause of AIH.

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Introduction

Autoimmune hepatitis (AIH) is a chronic necroinflammatory disorder of the liver of suspected autoimmune origin that can lead to end-stage liver disease and hepatic failure [1—3]. The mean incidence in Northern Europe is 1—2 per 100,000 person-year [4,5]. Women are more often affected than men (3.6:1) and all ethnic groups are equally affected [4,5]. AIH is characterized by elevated levels of serum autoantibodies and typical histopathological findings [1—3], as well as by hypergammaglobulinemia and response to immunosuppressive drugs [4,5]. The clinical presentation of AIH is protean and the clinical course may be characterized by fluctuating periods of increased and decreased activity. The pathogenesis of AIH remains unclear [5].

A working model for AIH pathogenesis postulates that environmental triggers, a failure of immune tolerance mechanisms, and a genetic predisposition collaborate to induce a T cell-mediated immune attack upon liver antigens, leading to a progressive necroinflammatory and fibrotic process in the liver [6,7]. The role of T cells in AIH establishment is supported by intrahepatic T cell population changes and AIH development in immunodeficient mice after receiving transfer of Traf6ΔTEC mice liver T cells, which exhibited all the histological and immunological characteristics of human AIH in their liver inflammation [8]. Such roles have been clearly documented in AIH-2, where CD4 and CD8T cells target a series of epitopes on CYP2D6, resulting in hepatocyte damage [9,10]. Two HLA DRB1*0301 restricted epitopes on SLA have been defined by Mix et al. [11] through the immunization of HLA DRB1*0301 (DR3) transgenic mice with recombinant SLA and confirmation with tetramer staining in HLA DR3-positive Northern European AIH patients. A previous study by our group have confirmed the relationship between cellular immune responses to SLA and severity of liver damage [12].

Nevertheless, the positive rate of anti-LKM and anti-SLA is low in patients with AIH in China [13,14] and more data are needed regarding autoantigen-specific T cell responses. In our previous work, a bifunctional mitochondrial enzyme, fumarate hydratase (FH), was found to be differentially expressed in the serum of patients with AIH compared with patients with viral hepatitis and other autoimmune diseases [13]. The specific involvement of FH-specific T cell response is currently unknown.

Therefore, this study aimed to investigate whether FH-specific T cells can be detected in peripheral blood of patients with AIH and are associated with disease progression. We synthesized overlapping peptides covering the sequence of FH and used ELISPOT assays to study the FH-specific T cell response in patients with AIH. The relationship between FH-specific T cell response and disease activity was analyzed. The results may help understanding the role of FH-specific T cells in the pathogenesis of AIH.

Materials and methods

Study design

This was a prospective study of 42 consecutive patients admitted to the clinical study center of autoimmune liver disease of our Hospital, Capital Medical University (China) between January 2011 and December 2014.

The study was approved by the ethics committee of our Hospital. Each patient provided a written informed consent.

Subjects

The inclusion criterion was: diagnosis of AIH, primary biliary cirrhosis (PBC), or chronic virus hepatitis. The exclusion criteria were:

- acute hepatitis virus infection;
- drug- and alcohol-induced hepatitis;
- any other autoimmune disease, such as systemic lupus erythematosus or rheumatoid arthritis;
- pregnancy;
- or treated with immune suppressors within the previous year.

Fourteen healthy controls were recruited during routine outpatient visits. Thirty patients with chronic hepatitis and 20 patients with primary biliary cirrhosis (PBC) were also recruited during routine outpatient visits.

The diagnosis of AIH was based on clinical, biochemical, and histopathological findings, as suggested by the simplified diagnostic criteria by Hennes et al. [15].

Clinical assessment

Remission was defined according to the current AASLD guidelines as normalization of serum transaminase and IgG level and absence of clinical symptoms [4]. Liver biopsies from 13 AIH patients were available at the time of the study and the severity of liver disease was scored as the histology activity index (HAI). Liver biopsies from others AIH patients were obtained far from the time of investigation and were only used for diagnosis but not for the analysis of relationship between HAI and T cell response.

Peripheral blood mononuclear cells (PBMC) separation and storage

Experiments were performed using either freshly separated or cryopreserved (trypan blue viability > 95%) PBMCs that were separated by Ficoll-Hypaque density gradient (Lymphoprep, Nycomed Pharma AS, Oslo, Norway) according to the manufacturer's instructions. Cryopreserved PBMCs were stored in liquid nitrogen for < 3 years before the study. Preliminary experiments using cell preparations from the same patients tested before and after cryopreservation showed no significant differences in cell viability and the ability to produce interferon (IFN)-γ.

Detection of autoantibodies

Anti-nuclear autoantibody, SMA, LKM-1, and antimitochondrial antibodies were detected by indirect immunofluorescence on cryostat sections of HEp-2 cells, monkey and rat liver, kidney, and stomach (Euroimmun, Luebeck, Germany)

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