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Effect of CD4+CD25+ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis

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Abstract

Background and aims: CD4 T lymphocytes constitutively expressing the IL-2-receptor α-chain (CD25) (T-regs) are central to self-tolerance maintenance, preventing the proliferation and effector function of autoreactive T-cells. In autoimmune hepatitis T-regs are defective in number but maintain the ability to suppress IFNγ production by CD4+CD25− T-cells. We have studied the ability of CD4+CD25+ (T-regs) to regulate proliferation and cytokine production by CD8 T-cells in patients with autoimmune hepatitis at diagnosis and during remission.

Methods: Twenty-five patients were studied. T-regs were purified from PBMCs by CD4 negative selection followed by CD25 positive selection, using immunomagnetic beads. The ability of T-regs to suppress CD8 T-cell proliferation was assessed by ^{3}H-thymidine incorporation; their ability to regulate cytokine production by intracellular cytokine staining.

Results: We found that T-regs are unable to regulate CD8 T-cell proliferation and cytokine production in patients studied at diagnosis, while they suppress CD8 T-cell proliferation and induce an elevation of IL-4 producing CD8 T-cells in patients during drug-induced remission.

Conclusion: Inability of T-regs to regulate CD8 T-cell function at diagnosis may contribute to the initiation of autoimmune liver damage. The ability of T-regs to regulate CD8 proliferation and IL-4 production during drug-induced remission suggests a role for immunosuppressive treatment at reconstituting T-regs function.

Keywords: Hepatitis; Autoimmunity; Cytokine; IL-4; Immunosuppressive

1. Introduction

Autoimmune hepatitis is an inflammatory liver disease characterised by presence of high transaminases, circulating autoantibodies, hypergammaglobulinaemia, histological evidence of interface hepatitis and response to immunosuppressive treatment [1,2]. There are two types of autoimmune hepatitis: one positive for anti-nuclear (ANA) and/or smooth muscle (SMA) antibodies [3], and one positive for liver kidney microsomal antibody type 1 (LKM1) [4,5]. An ANA/SMA positive overlap syndrome between autoimmune hepatitis and

Abbreviations: IL-2R, interleukin 2 receptor; T-regs, regulatory T-cells; ANA, anti-nuclear antibodies; SMA, anti-smooth muscle antibodies; LKM1, liver kidney microsomal antibody type 1; AST, aspartate aminotransferase; SLA, soluble liver antigen; IFNγ, interferon γ; IgG, immunoglobulin G; NV, normal value.

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sclerosing cholangitis is also observed in young patients [6].

A T-cell mediated immune response is thought to play a major role in the causation of autoimmune liver damage. In addition to CD4 T-cells, there is growing evidence suggesting a role for CD8 T-cells. CD8 positive T-cell clones, specific for asialoglycoprotein receptor, were readily established in patients with autoimmune hepatitis by Wen et al. [7]. In patients with another liver autoimmune disease, primary biliary cirrhosis, a CD8 response against the E2 components of pyruvate dehydrogenase complex, the main autoantigen in PBC, has been identified and extensively characterised [8,9].

The mechanisms leading to the breakdown of self-tolerance and consequent development of autoimmune disease are unclear, though a failure of homeostatic processes, normally keeping the response against self-antigens under control, is probably involved. Among different T-cell sub-populations known to participate in this homeostatic process, such as natural killer T-cells (NKT), T helper 3 (Th3), T regulatory 1 (Tr1), CD8+CD28− and γδ T-cells, CD4+ lymphocytes constitutively expressing the interleukin 2 receptor (IL-2R) α-chain (CD25) have emerged as a major immunoregulatory population [10]. In the experimental animal their removal is associated with the onset and development of autoimmune disease, such as insulin dependent diabetes, gastritis and autoimmune thyroiditis [11]. Their precise mechanism of action, possibly involving a contact with the target cells or the release of immunoregulatory cytokines, such as transforming growth factor β (TGFβ) and interleukin 10 (IL-10), is still under investigation [12–16]. Apart from CD25, additional markers expressed by T-regs include the glucocorticoid induced tumour necrosis factor receptor (GITR), the cytotoxic T lymphocyte associated protein 4 (CTLA-4), CD62L and the forkhead/winged helix transcription factor *FOXP3*, which is considered a key regulatory gene required for the development and functional activity of CD4+CD25+ T-regs [17,18]. CD4+CD25+ T-regs are able to prevent the proliferation and effector function of autoreactive T-cells. Results from our group have shown that in patients with autoimmune hepatitis these cells, even though decreased in number and impaired in their ability to expand, are still able to inhibit interferon γ (IFNγ) production by CD4+CD25− T-cells [19].

CD4+CD25+ T-regs have also been reported to down-regulate the production of IFNγ by CD8 T-cells in a murine model and in humans [20,21]. In addition to an IFNγ producing subset, another CD8 T-cell sub-population has been recently described that produces IL-4 [22], a cytokine with immune down-regulating properties [23,24]. To date no studies have investigated the ability of T-regs to regulate CD8 T-cell cytokine response in patients with autoimmune hepatitis.

The aim of the present study was to investigate the ability of T-regs to regulate proliferation, IFNγ and IL-4 production by purified CD8 T-cells from patients with autoimmune hepatitis at different stages of disease activity.

2. Patients and methods

2.1. Patients

Twenty-eight patients with autoimmune hepatitis were studied: 23 ANA/SMA and 5 LKM1 positive. All had histological features of interface hepatitis at diagnosis. Radiological bile duct changes on retrograde cholangiography, characteristic of sclerosing cholangitis were present in 5 ANA/SMA positive patients, who were diagnosed as having overlap syndrome. Twenty-one patients were female (17 ANA/SMA positive, 4 LKM1 positive). Median age was 13.3 years, ranging from 7 to 26 years. Fourteen patients (13 ANA/SMA positive and 1 LKM1 positive) were studied at disease presentation, while 19 (15 ANA/SMA positive and the 4 LKM1 positive) were studied during remission (defined as normal transaminase levels) on immunosuppressive treatment. Five patients (ANA/SMA positive) were investigated both at diagnosis and remission. In the patients studied at diagnosis median AST was 186 IU/l (range 55–4830; normal value [NV] < 50 IU/l), bilirubin 85 μmol/l (range 5–306; NV < 20 μmol/l) and immunoglobulin G (IgG) 26 g/l (range 11–62; NV 6.5–17 g/l). In the patients studied during remission AST was 29 IU/l (range 18–45), bilirubin 9 μmol/l (range 5–37) and IgG 10 g/l (range 7–23). In ANA/SMA positive patients the median titres of ANA and SMA were 1/320 (range 1/40–1/640) and 1/640 (range 1/80–1/1280) at presentation and 1/40 (range 1/20–1/320) and 1/20 (range 1/20–1/640) during treatment. In patients with type 2 autoimmune hepatitis, the titre of LKM1 was 1/10 240 in the patient studied at disease presentation while in the four patients studied during remission it was 1/160 and 1/40 in two and negative in the remaining. The 19 patients studied during treatment were receiving prednisolone (2.5–5 mg daily) with or without azathioprine (1–2 mg/kg/day). Of the three assays presented in the current work, at least two were performed in 20 patients; three assays were performed in the remaining 8 patients. All patients were negative for HCV RNA by PCR as well as for serological markers of hepatitis B and A, and for IgM to EBV and CMV. Fifteen healthy subjects, 10 female and 5 male, were investigated as controls (median age 29 years, range 13–38 years). The study was approved by the Ethical Committee of King's College Hospital, London, UK.