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T-regs in autoimmune hepatitis-systemic lupus erythematosus/mixed connective tissue disease overlap syndrome are functionally defective and display a Th1 cytokine profile

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ABSTRACT

Autoimmune hepatitis (AIH), a severe hepatopathy characterized by hypergammaglobulinaemia, autoantibodies and interface hepatitis, is occasionally associated with systemic autoimmune manifestations [systemic lupus erythematosus (SLE); mixed connective tissue disease (MCTD)]. In both AIH and SLE/MCTD numerical and/or functional impairment of regulatory T-cells (T-regs) is believed to favour autoimmunity. To investigate whether immune-tolerance breakdown profiles differ in patients with AIH and SLE/MCTD, isolated AIH or systemic autoimmunity, we studied phenotypic and functional features of T-regs in 10 patients with AIH-SLE/MCTD, 22 with AIH, 12 with SLE and 20 healthy subjects. Compared to health, $CD4^{pos}CD25^{pos}$ cells were decreased in number and expressed high levels of the CD127 activation marker in all three disease groups; in AIH-SLE/MCTD and in SLE they displayed low levels of FOXP3. In AIH-SLE/MCTD, they also contained a high proportion of IFNγ positive cells, indicating a Th1 profile. Similarly, in AIH-SLE/MCTD, $CD4^{pos}CD25^{pos}CD25^{high}$ T-regs were reduced in number and contained an increased proportion of activated $CD127^{pos}$ and $IFN\gamma^{pos}$ cells. Such skewing towards a Th1 profile was also present at effector level, as a high frequency of IFNγ-producing cells was observed within AIH-SLE/MCTD $CD4^{pos}CD25^{neg}$ responder cells. Impairment in suppressor function both of $CD4^{pos}CD25^{pos}$ cells and $CD4^{pos}CD25^{pos}CD127^{neg}$ T-regs was observed in all three autoimmune conditions, but while addition of $CD4^{pos}CD25^{pos}CD127^{neg}$ T-regs decreased $CD4^{pos}CD25^{neg}$ responder cell proliferation in healthy subjects and partially in AIH patients, it had no effect in AIH-SLE/MCTD and SLE patients.

In conclusion, in AIH-SLE/MCTD T-regs display a distinctive phenotypic and functional signature, characterized by marked activation, elevated IFNγ production and by a profound impairment of suppressive function, suggesting that multiple autoimmune manifestations may derive from a complex defect of immune-regulation.

1. Introduction

Defective immune-regulation plays a key role in the development of autoimmune disorders in both experimental and clinical settings. $CD4^{pos}CD25^{high}FOXP3^{pos}$ regulatory T-cells (T-regs) are the main players in the maintenance of immune homeostasis by controlling autoreactive immune responses to self-antigens [1,2]. Numerical and functional T-reg impairment has been reported in autoimmune hepatitis (AIH) [3–5], an inflammatory liver disorder, characterized by hypergammaglobulinaemia, seropositivity for circulating autoantibodies and interface hepatitis on histology. T-reg impairment in AIH plays a permissive role allowing effector CD4 and CD8 T-cells to target the liver. Thus, T-regs isolated from AIH patients fail to control the proliferation and IFNγ production/secretion by CD4 and CD8 effector T lymphocytes, this defect being more marked at disease presentation than during drug-induced remission [3,4].

Abbreviations: AIH, autoimmune hepatitis; SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; anti-LKM1, anti-liver kidney microsomal type 1 antibody; AST, aspartate amino transferase.

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In up to 20% of cases AIH is associated with other autoimmune disorders, including arthritis, type 1 diabetes, ulcerative colitis and thyroiditis [6]. AIH is occasionally associated with systemic autoimmune manifestations (affecting kidneys, skin, synovium and/or central nervous system) in the form of classical systemic lupus erythematosus (SLE) or mixed connective tissue disease (MCTD). A wealth of studies conducted both in mice and patients have demonstrated numerical and/or functional impairment of T-regs in SLE, a condition characterized by loss of tolerance to self-nuclear antigens [7]. Defective T-regs have been reported also in MCTD, a disorder that shares with SLE B and T-cell autoreactivity to RNA-associated molecules, including small ribonucleoprotein particles, RNA polymerases, SS-A/Ro, SS-B/La and tRNA synthetases [8,9]. The prevalence of T-regs in (New Zealand Black × New Zealand White) F_1 (BWF_1) and in (SWR × New Zealand Black)F_1 (SNF_1) lupus-prone mice is lower than in non-autoimmune strains [10], while depletion of T-regs through thymectomy leads to expansion of autoreactive T-cells and diffuse organ inflammation in SNF_1 mice [11]. Subsequent studies have shown that in MRL/lpr lupus-prone mice T-regs display a reduced suppression of proliferation and pro-inflammatory cytokine secretion by effector cells [12]. In line with murine studies, investigations in human SLE, have shown that natural T-regs are numerically decreased or functionally defective during active phases of the disease in adult [13–16] and paediatric patients [17]. With regard to T-reg function, Valencia and co-workers have reported that $CD4^{+}CD25^{high}$ T-regs isolated from patients with active SLE fail to control proliferation and cytokine secretion of effector T-cells in vitro, whereas T-regs isolated from patients with inactive disease suppress as efficiently as T-regs from healthy subjects [18]. In a study including 48 patients with MCTD, Baráth and colleagues report that the percentage and the absolute number of $CD4^{pos}CD25^{high}$ T-regs is lower than in healthy subjects and that T-reg numerical impairment is more marked when the disease is active [9].

We have investigated whether phenotypic and functional features of T-regs in patients with concomitant AIH and SLE or MCTD differ from those of patients with isolated AIH or isolated SLE.

2. Materials and methods

2.1. Patients and controls

Ten patients with AIH and concomitant SLE/MCTD manifestations (2% of all AIH children in our database), were studied. Seven patients fulfilled at least four of the diagnostic criteria for SLE [19], while three fulfilling fewer than four SLE criteria were diagnosed as having MCTD (Table 1). Patients with AIH and SLE or MCTD are henceforth referred to as AIH-SLE/MCTD. Nine patients had AIH type 1 (AIH-1) and were positive for anti-nuclear (ANA) and/or smooth muscle (SMA) antibody; the remaining patient had type 2 AIH (AIH-2) and was positive for both anti-liver-kidney-microsomal antibody type 1 (anti-LKM-1) and ANA. A liver biopsy performed at the time of or close to diagnosis showed histological features of interface hepatitis in all. All patients were on immunosuppressive treatment at the time of study, seven being investigated while in remission (i.e. normal transaminase levels), two during an episode of relapse and one 11 days after diagnosis. Twenty-two patients with AIH-1 only were studied as pathological controls. Fifteen were in remission, 6 in relapse and 1 at diagnosis. All AIH-SLE/MCTD and AIH patients were receiving prednisolone (2.5–5 mg daily at remission and 1–2 mg/kg/day at relapse/diagnosis) with or without azathioprine (1–2 mg/kg daily). Twelve patients with SLE only were studied as additional pathologic controls (Table 1). Demographic and laboratory data of AIH-SLE/MCTD, AIH and SLE patients are summarised in Table 2. Twenty healthy subjects (18 females; median age: 3.3 years, range 21.7–55.4 years), recruited from King's College Hospital staff members, served as normal controls. Written consent was obtained from each subject. The study was approved by the Ethics Committee of King's College Hospital, London, UK.

2.2. Cell separation

Peripheral blood mononuclear cells (PBMCs) were obtained as previously described [3]. Mononuclear cell viability, determined by Trypan blue exclusion, exceeded 98%.

2.3. Flow cytometry

T-reg phenotype was determined by flow cytometry using APC-Cy7-conjugated anti-CD4, PE or APC-conjugated anti-CD25 and FITC-conjugated anti-CD127 monoclonal antibodies (all from BD Bioscience Discovery Labware, Oxford, UK). Expression of FOXP3, the T-reg lineage specific transcription factor [20,21], was evaluated by intracellular staining [4] using APC-conjugated anti-human FOXP3 (clone PCH101, eBioscience, Hatfield, UK) monoclonal antibody. Frequencies of cytokine-producing cells within $CD4^{pos}CD25^{pos}$ and $CD25^{high}$ T-reg fractions and within $CD4^{pos}CD25^{neg}$ cells were determined by intracellular cytokine staining, following cell exposure to Brefeldin A for 5 h, fixation, permeabilization and staining with FITC-conjugated anti-interleukin 4 (IL-4) (BD Bioscience) and anti-IL-17 (eBioscience) and APC-conjugated anti-IFNγ (BD Bioscience) monoclonal antibodies. Cells were acquired using a BD FACSCanto II (Becton Dickinson Immunocytochemistry Systems, San José, CA); FACSDiva software was used for analysis. A minimum of 1×10^4 gated events was acquired for each sample.

2.4. $CD4^{pos}CD25^{pos}$ and $CD4^{pos}CD25^{neg}$ cell purification

$CD4^{pos}CD25^{pos}$ and $CD4^{pos}CD25^{neg}$ cells were purified from PBMCs using immunomagnetic beads (Dynal Invitrogen, Oslo, Norway) as previously described [5]. $CD4^{pos}CD25^{pos}$ and $CD4^{pos}CD25^{neg}$ T-cell purity exceeded respectively 95% and 90% (range 92%–97%). $CD4^{pos}CD25^{pos}CD127^{neg}$ T-cells [22], were purified from $CD4^{pos}CD25^{pos}$ cells following incubation with PE-conjugated anti-CD127 monoclonal antibody (BD Bioscience) for

Table 1
SLE manifestations in AIH-SLE/MCTD and SLE patients.

	Arthritis	Mouth ulcers	Malar rash	Photosensitivity	Discoid	Serositis	Renal	Central nervous system	Haematological	ANA	Anti-dsDNA
AIH-SLE ($n = 7$)	6	1	4	0	2	2	2	0	4	7	5
AIH-MCTD ($n = 3$)	2	0	1	0	0	1	0	0	0	3	0
SLE ($n = 12$)	11	2	4	3	1	1	5	0	9	12	8

AIH: autoimmune hepatitis.
SLE: systemic lupus erythematosus.
MCTD: mixed connective tissue disease.
ANA: anti-nuclear antibody.
dsDNA: anti-double stranded DNA antibody.