Elevated CD4⁺/CD25⁺ T cell frequency and function during acute hepatitis C presage chronic evolution

Hepatitis C virus (HCV) determines an acute hepatitis evolving to persistent infection in 50–80% of patients.¹ Different mechanisms have been proposed to explain disease evolution, including viral escape, failure of the T helper immune network, and host genetic factors.² Among CD4⁺ T cell subsets, lymphocytes expressing constitutively CD25 (interleukin 2 receptor alpha chain), namely T regulatory cells (Treg), appear to play a critical role in controlling chronic evolution of HCV mediated liver diseases.³ Here we investigate the frequency and functional activity of CD3⁺/CD4⁺/CD25⁺ Tregs in acute HCV infection in relation to its evolution over time.

The study was approved by the local ethics committee and included 16 consecutive patients with acute hepatitis C (AHC), 15 consecutive patients with acute hepatitis A (AHA), and 10 healthy donors (table 1). Criteria for diagnosis of acute hepatitis C were in accordance with previous reports.⁴ At the start of the study, our hospital did not offer interferon α treatment to acute patients, and even when treatment became available, none of the patients signed the written informed consent for treatment but agreed to be followed up. No patient had hepatitis B virus, cytomegalovirus, Epstein-Barr virus, or human immunodeficiency virus infection markers, a history of alcohol abuse, or evidence of autoimmune liver disease (antinuclear antibody, antimitochondrial antibody, anti-liver/kidney microsomal antibodies).

Blood samples were collected at admission to hospital (T0), which occurred at least one week after the first clinical symptoms, and retested six months later (T6). HCV-RNA was assayed qualitatively and quantitatively using Amplicor Roche system 2.5 with cut off points of 50 and 600 IU/ml, respectively. T lymphocytes subsets from peripheral blood were counted by multicolour flow cytometry.^{5,6} For functional studies, Tregs were isolated by positive selection using antibody coated magnetic beads (Dynal Treg Kit; Dynal, Oslo, Norway). Tregs suppressor activity was measured as described previously.⁷ The Mann-Whitney U test (two tailed) and Spearman's test were used for statistical analysis.

An increase in Treg frequency and suppressor activity was observed in 12 (all genotype 1) of 16 AHC patients and persisted for six months after diagnosis (T6), by which time HCV-RNA was positive, assessed both qualitatively quantitatively, and transaminase levels exceeded twice the upper limit of normal. No significant increase in Tregs was observed in AHA patients (table 1, fig 1). Four of 16 HCV infected patients who showed no significant increase in Treg frequency or function at the time of admission to hospital (patient 1 8% and 80 cells/mm³ genotype 2a/2c; patient 2 7% and 72 cells/mm³ genotype 1b; patient 3 8.5% and 82 cells/mm³ genotype 3a/3c; patient 4 9% and 85 cells/mm³ with genotype 2a/2c) went on to clear the virus (negative HCV-RNA) and had normal transaminase levels six months after acute hepatitis.

CD4⁺/CD25⁺ are known to be increased in patients with chronic hepatitis C but not in those who resolve the infection.³ Our findings

Table 1 Patient characteristics

Parameter	HCV-T0	HCV-T6	HAV	HD
Age (y) (mean (SD))	42 (3)	40 (2)	32 (5)	35 (6)
Sex (M/F)	10/6	12/12	11/4	7/3
ALT level (U/l <40)	1425 (539)	100 (50)	1418 (543)	<40
AST level (U/l <40)	892 (305)	84 (24)	851 (221)	<40
HCV-RNA (IU/ml (mean (SD)))	310 (232) × 10 ³	Positive	na	na
Genotype				
1a/1b	12/16	11/12	0	
Non-1	4/16	1/12		
Mode of infection				
Needlestick	1/16		0	
Surgery procedure	2/16		0	
Alimentary	0		15/15	
Unclear	13/16		0	
Immunological parameters				
CD3 ⁺ CD4 ⁺	956 (297)†**	892 (103)	725 (200)**	772 (297)**
CD3 ⁺ CD4 ⁺ CD25 ⁺	130 (50)†**	111 (12)	46 (26)**	46 (13)**
CD3 ⁺ CD4 ⁺ CD25 ⁺	13.5 (3)‡**	11.2 (2)	6.3 (1)**	6 (1)**
T regulatory functional assay¶				
Ratio 1:2¶	100§**	92	80	79
Ratio 1:20	99**	85**	70	55

HCV-T0, patients with hepatitis C virus at time 0; HCV-T6, patients hepatitis C virus after six months; HAV, patients with acute hepatitis A; HD, healthy donors; na, not applicable; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Statistically significant (Mann-Whitney U test, $p < 0.01$).

†Total count number (cells/mm³; values are mean (SD)).

‡Percentage.

¶Purified CD3⁺ CD4⁺ CD25^{bright} (Treg) were cocultured at different ratios (1:2 and 1:20) with autologous CD3⁺ CD4⁺ CD25⁻ lymphocytes in the presence of CD3/CD28 conjugated beads (Dynal Biotech, Oslo, Norway).

§Percentage suppression was calculated assuming 0% counts per minutes (cpm) incorporated by 20 000 CD4⁺ CD25⁻ purified T cells stimulated with CD3/CD28 coated beads (Dynal) in the absence of Treg. Mean (SD) of the absolute cpm found in healthy controls, HCV, and HAV was 25 000 (5000), 24 000 (6000), and 22 000 (4000), respectively.

Figure 1 CD4⁺ CD25⁺ Treg levels in representative patients with acute hepatitis C virus (HCV) and controls. CD4⁺ CD25⁺ Treg levels were higher in acutely HCV infected patients (A) who underwent chronic evolution in comparison with acutely HCV infected patient who resolved the infection (B). (C) Patient with hepatitis A virus (HAV). (D) Healthy donor.

indicate that an increase in Treg number and function, if present in the acute hepatitis phase of the infection, predicts a chronic evolution and persists over time. Interestingly, all patients with elevated Tregs and chronic evolution harboured genotype 1 virus, suggesting a possible influence of that subset on chronic evolution,⁸ perhaps through Treg triggering. Whether the elevation in regulatory T cells reported here is just a predictor of chronic evolution of HCV infection or a promoter of HCV infection chronicity, remains to be investigated.

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Thalidomide in refractory haemorrhagic radiation induced proctitis

One of the most frequently encountered late gastrointestinal sequelae of abdominopelvic radiation therapy is radiation proctitis, occurring in 12.5–19% of patients.^{1,2} Treatment options range from drug therapy, including spasmolytics, steroids, mesalasin, and endoscopic treatment comprising argon plasma coagulation (APC), laser coagulation, and formalin application, to hyperbaric oxygen therapy and finally surgical intervention.^{3–5} We describe a patient with severe refractory haemorrhagic radiation proctitis requiring frequent blood transfusions who was successfully treated with low dose oral thalidomide.

Case report

A 78 year old female presented with a three month history of intermittent rectal blood loss. Her medical history revealed cervical cancer stage IIIB two years earlier. She had been treated with a multimodal regimen comprising chemotherapy (cisplatin 40 mg/m² at weekly intervals for six weeks) and radiation therapy (EBRT 50.4 Gy in 28 fractionated doses of 1.8 Gy plus two sessions of brachytherapy). Physical examination was unremarkable except for brisk red blood at digital rectal examination. Laboratory investigation yielded microcytic anaemia (haemoglobin 4.7 mmol/l). At colonoscopy, the distal 10–15 cm of the rectal mucosa appeared circumferentially abnormal, being highly friable and slightly bleeding, interspersed with multiple telangiectasias, compatible with radiation proctitis. Despite consecutive treatment, including topical mesalazin and/or steroid enemas, epsilon-aminocaproic acid (both orally and later topical), and several sessions of APC, no long term beneficial effect was achieved. The patient received blood transfusions at a rate of 2–3 packed cells per 2–3 weeks. Surgical options were discussed but refused. After written informed consent, low dose thalidomide was started at 50–100 mg/day. Three weeks later her haemoglobin level had remained stable after the last transfusion. Currently the patient is doing well after three months without any blood transfusions.

As illustrated by this case history, treatment of haemorrhagic radiation induced proctitis can be difficult. Because of the severe course, despite various treatment regimens and refusal of surgical intervention, other treatment options were considered. Application of formalin has been reported to effectively reduce rectal bleeding. Yet serious local adverse effects have been described in up to 19% of cases, such as painful rectal ulceration, anorectal stricture formation, and anal incontinence. Although these complications may represent the natural sequelae of radiation induced proctitis, formalin application may have contributed to the deterioration due to its direct effect on mucosal blood flow.^{3–6}

We treated this patient with low dose thalidomide with remarkable results. Thalidomide is a potent inhibitor of (neo)angiogenesis, mediated by suppression of vascular endothelial growth factor (VEGF).⁷ Bauditz *et al.* reported cessation of bleeding in otherwise refractory angiodysplasias and bleeding Crohn's disease, presumably as a result of decreased levels of VEGF during thalidomide treatment.⁸ Although the exact role of VEGF in radiation induced mucosal injury needs to be elucidated, the temporal relationship between thalidomide therapy and cessation of bleeding in our patient is highly suggestive of a causal relationship. Experimental data, suggestive of a potential role for VEGF modulation, indicate that VEGF expression is upregulated in normal rat brain after experimental intraoperative radiation therapy.⁹ Moreover, radiation induced hypoxia may perpetuate late normal tissue injury leading to progressive occlusive vasculitis, collagen deposition, and fibrosis in the presence of increased local transforming growth factor β , VEGF, and CD31, suggesting hypoxia mediated activation of the profibrinogenic and proangiogenic pathways.¹⁰

In conclusion, our preliminary experience suggests that thalidomide might be an effective treatment option in patients with refractory haemorrhagic radiation proctitis. As we are the first—to the best of our knowledge—to report this potential therapeutic effect obviating the need for surgical intervention, further clinical studies are warranted, defining the effectiveness and side effects in a larger group of patients.

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