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Dysregulated expression of T-helper cell responses and susceptibility to infections in high-risk liver transplant recipients

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ABSTRACT

Background: Liver transplant recipients requiring dialysis have poor outcomes including higher risk of infection, and allograft rejection. The role of T-helper cell cytokine responses in the pathogenesis of infections in these patients has not been fully defined.
Methods: Cases were 11 dialyzed liver transplant recipients. Controls (2 for each case) were patients who were transplanted next to the case but did not require dialysis at any time before or after transplantation. Cytokine responses were assessed in sera collected immediately before transplantation. Data were analyzed for candidate cytokines for pro-inflammatory Th1 (IL-1β, IL-12p70, and IFN-γ), and Th17 (IL-12p40, IL-17), and anti-inflammatory Th2 phenotypes (IL-4, IL-5, IL-10, and IL-13).
Results: Cases were more likely to have an increase in any of the Th1 or Th1 and Th17 cytokines than the controls ($p=0.016$ and $p=0.04$, respectively). Major infections developed in 27% of the study population; these included 46% of the cases and18%, of the controls ($p=0.09$). Patients with infections vs. those without these were more likely to have an increase in any of the Th2 cytokines ($p=0.005$). CMV viremia occurred in 30% of the patients and was significantly associated with Th1 responses even when adjusted for CMV recipient/donor serostatus or any major infection (OR 3.2, 95% CI 0.96–10.73, $p=0.05$).
Conclusions: Requirement of dialysis was characterized by a state of heightened expression of inflammatory responses. However, patients developing infections preferentially expressed Th2 phenotype that may act as a negative regulator of protective inflammatory responses. An enhanced expression of inflammatory mediators may serve to promote CMV infection in liver transplant recipients.

1. Introduction

Liver transplant recipients requiring dialysis are at high risk for poor outcomes after transplantation [1–8]. Infections occur in 85%–91% of the liver transplant recipients requiring dialysis compared to 38%–41% in those not requiring dialysis [1,5]. In addition, the need for dialysis in liver transplant recipients confers a higher risk of biopsy-proven rejection and lower allograft survival [4,5,7]. Furthermore, compared to liver transplant recipients not on dialysis, those on dialysis have a significantly higher mortality (35%–55% vs. 4%–26.8%) [1,2,4,6,7]. In one study, nearly half of the deaths in dialyzed liver transplant recipients were related to miscellaneous or unreported causes(53%) followed by cardiovascular events (20.8%) and infection (20.3%) [6]. It is not clear why dialysis has such a devastating impact on the post-transplant course.

Host defense against bacterial and fungal infections is critically regulated by T-helper cytokine response. Th1 and other pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interferon-γ (IFN-γ), and interleukin (IL)-2 confer protection against pathogens [9–11]. However, these cytokines are also the primary mediators of allograft rejection in transplant recipients [12,13]. On the other hand, Th2 cytokines, such as IL-4 or IL-10 inhibit Th1 responses and compromise the protective host immunity [14,15], but promote transplant tolerance [16]. In addition T-helper cells, in the presence of transforming growth factor (TGF-β) and IL-6 can differentiate to Th17 cells which secrete IL-17 and have recently been recognized to induce potent inflammatory responses [17,18]. Dialyzed liver transplant recipients developing post-transplant infection are not neutropenic [5]. Whether susceptibility of these patients to opportunistic infections results from differential expansion of Th cell subsets and an altered pattern of Th cytokine synthesis however, is not known.

A number of reports have shown a relationship between cytokines and allograft rejection in transplant recipients [12,13,19]. However, few have examined the association between cytokine responses and

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Table 1
Demographics characteristics of dialyzed transplant recipients and the controls

Factors	Cases[a]	Controls[a]
Patient number	11	22
Mean age, years (median, range)	51 (52, 34–60)	51 (53, 36–68)
Gender, male, *n* (%)	10 (91)	21 (95)
Underlying liver diseases[b], *n* (%)		
Hepatitis C virus infection	7 (64)	16 (73)
Alcoholic liver disease	2 (18)	6 (27)
Hepatitis B virus infection	1 (9)	0 (0)
Metabolic disease	1 (9)	2 (9)
Cryptogenic liver disease	1 (9)	2 (9)
Primary biliary cirrhosis	1 (9)	0 (0)
Hepatocellular carcinoma	1 (9)	5 (23)
Child–Pugh score, median (range)	13(10–15)	12 (7–15)
Cytomegalovirus serostatus, *n* (%)		
R−/D−	1 (9)	1 (5)
R+/D−	7 (64)	5 (23)
R−/D+	1 (9)	6 (27)
R+/D+	2 (18)	10 (45)
Mean operation time, h	12.5	11.3
Median blood loss, units (range)	13 (4–34)	9 (2–23)

[a] All comparisons of the factors between the cases and controls were insignificant.
[b] May have more than one underlying liver disease.

opportunistic infections in solid organ transplant recipients [20,21]. Thus, the present study aimed to assess the role of T cell cytokine responses in the pathogenesis of infections in liver transplant recipients requiring dialysis.

2. Patients and methods

Patients compromised consecutive liver transplant recipients at the VA Pittsburgh Health Care System from 1996 to 2007 who required dialysis post-transplantation. Two controls were selected for each case. The control patients were transplanted next to the case but did not require dialysis at any time before or after transplantation. Since immunosuppressive agents employed after transplantation lead to a shift toward Th2 in all patients, cytokine responses were assessed in the sera collected immediately prior to transplantation. Data regarding age at transplantation, gender, underlying liver disease, donor and recipient CMV serostatus, Child–Pugh score, operation time, blood loss, and bacterial or fungal infections were analyzed.

Infections occurring within 90 days of transplantation were considered for analysis. Bacterial infections were defined as per the criteria proposed by the Centers for Disease Control and as previously reported in liver transplant recipients [22,23]. Fungal infections were defined as previously reported [24]. All patients underwent surveillance monitoring at weeks 2, 4, 6, 8, 12, and 16 after transplantation using CMV antigenemia or TaqMan assay (since June 2006), a quantitative real-time DNA polymerase chain reaction (PCR) [25]. Patients with antigenemia level ≥1 or CMV DNA ≥15 copies/ml by Taqman-PCR were considered as having CMV viremia and received either oral ganciclovir or valganciclovir as preemptive therapy as previously reported [25]. The study was approved by the institutional review board of the Pittsburgh VA Healthcare System.

All patients received tacrolimus and low-dose prednisone as standard immunosuppressive regimen as previously described [1,5]. Mycophenolate mofetil was added if nephrotoxicity or neurotoxicity due to tacrolimus was suspected. Rejection episodes were treated with 1 g of methylprednisolone with or without a corticosteroid recycle (methylprednisolone administered in dosages tapered from 200 to 20 mg per day over 6 days).

Sera were frozen at −70 °C immediately upon collection and tested for cytokine responses in a single batch by an investigator (CS) who was unaware of the clinical status or the study group of the patients. Cytokine levels were quantified using the Bio-Plex Protein Array System and a Human Cytokine 27-plex Panel (Bio-Rad Laboratories, Hercules, CA). Briefly, anti-cytokine/chemokine antibody conjugated beads were added to individual wells of a 96-well filter plate and adhered using vacuum filtration. After washing, 50 μl of pre-diluted standards or samples were added and the filter plate was shaken at 300 rpm for 30 min at room temperature. Thereafter, the filter plate was washed and 25 μl of pre-diluted multiplex detection antibody was added for 30 min. After washing, 50 μl of pre-diluted streptavidin-conjugated phycoerythrin was added for 10 min followed by an additional wash and the addition of 125 μl of Bio-Plex assay buffer to each well. The filter plate was analyzed using the Bio-Plex Protein Array System and concentration of each cytokine was determined using Bio-Plex Manager Version 3.0 software. Cytokine levels were adjusted for total proteins and expressed as pg/ml. Data are presented for candidate cytokines representative of Th1 response (IL-1β, IL-12p70, and IFN-γ), Th2 response (IL-4, IL-5, IL-10, and IL-13), Th17 response (IL-12p40 and IL-17), Th1 and Th17 response (IL-1β, IL-12p70,IL-12p40, IL-17, and IFN-γ), and inflammatory mediators (IL-1α, IL-1β, IL-6, and TNF-α).

2.1. Statistical analysis

Cytokine levels in dialyzed and non-dialyzed patients were compared using the rank sum test. Increased levels of cytokines were approximated by considering levels above the median for the entire group as being elevated. The numbers of estimated increased cytokine

Table 2
Cytokines responses in dialyzed patients vs. the controls

	Case	Control	OR	95% CI
Th1[a] (IL-1β, IL-12p70, and IFN-γ)				
0	0	11		
1	2	3	0.667	0.111–3.990
2	5	3	1.667	0.398–6.974
3	4	5	0.800	0.215–2.979
Th17[b] (IL-12p40 and IL-17)				
0	1	9	0.111	0.014–0.877
1	6	8	0.750	0.260–2.162
2	4	5	0.800	0.215–2.979
Th1 and Th17[c] (IL-1β, IL-12p70,IL-12p40, IL-17, and IFN-γ)				
0	0	7		
1	2	6	0.333	0.067–1.652
2	2	1	2.000	0.181–22.056
3	5	5	1.000	0.290–3.454
4	2	3	0.667	0.111–3.990
Th2[d] (IL-4, IL-13, IL10, IL-5)				
0	0	6		
1	2	6	0.333	0.067–1.652
2	5	4	1.250	0.336–4.655
3	2	3	0.667	0.111–3.990
4	2	3	0.667	0.111–3.990
Inflammatory mediators[e] (IL-1α, IL-1β, IL-6, and TNF-α)				
0	1	7	0.143	0.018–1.161
1	3	6	0.500	0.125–1.999
2	2	5	0.400	0.078–2.062
3	3	1	3.000	0.312–28.841
4	2	3	0.667	0.111–3.990

OR = odds ratio.
CI = confidence interval.
[a] $p=0.016$. Dialysis patients were more likely to have increases in Th1 cytokines; 44% (4/9) of patients with all 3 cytokines elevated were dialysis patients compared to 0% (0/11) with no increase in Th1 cytokine levels.
[b] $p=0.110$. No significant association between dialysis patients and the increased levels of Th17 cytokines.
[c] $p=0.049$. Dialysis patients were more likely to have increases in Th1 and Th17 cytokines; 40% (2/5) of patients with all 4 cytokines elevated were dialysis patients compared to 0% (0/7) with no increase in Th1 and Th17 cytokine levels.
[d] $p=0.115$. No significant association between dialysis patients and the increased levels of Th2 cytokine.
[e] $p=0.131$. No significant association between dialysis patients and the increased levels of inflammatory mediators.