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Research Article

Plasma transforming growth factor-beta 1 and anti-HLA IgM antibodies affect the functional preservation of kidney allografts

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Abstract

To elucidate which of the plasma cytokines, interleukin-2 and interferon-γ, interleukin-4, interleukin-10, and transforming growth factor-β1, is most closely associated with the progression of chronic renal allograft injury (CRAI), we analyzed plasma from 87 patients who had undergone kidney transplantation. When the patients were classified into two groups based on the level of plasma creatinine, the transforming growth factor-β1 level was significantly higher in the normal creatinine group than in the high creatinine group. When the patients showing CRAI were compared with patients without CRAI, the latter had a significantly higher plasma concentration of transforming growth factor-β1. These results suggest that plasma transforming growth factor-β1 helps to preserve the function of kidney allografts by suppressing the progression of CRAI. Finally, we analyzed anti-HLA class I and class II antibodies of both the IgG and IgM immunoglobulin classes in the patients' plasma. It was found that the high creatinine group showed a significantly higher frequency of IgM antibodies against HLA class I and/or class II than did the normal creatinine group, suggesting that anti-HLA IgM antibodies may be implicated in functional impairment of kidney allografts.

Keywords: Chronic Renal Allograft Injury; Plasma Cytokines; Transforming Growth Factor; Anti-hla IgM antibodies

Introduction

Chronic renal allograft injury (CRAI) is the most important problem to be overcome in modern kidney transplantation medicine [1]. The etiology of CRAI is multifactorial and involves both immunological and non-immunological mechanisms [2,3]. Although the non-immunological factors such as ischemia and arteriosclerosis increase the risk of CRAI, immune activation, especially toward allogeneic HLA, is thought to play a critical role in the initiation and progress of CRAI [4,5].

Immune responses, including those against alloantigens, are classified into two different modes: type 1 and type 2 helper T cell responses (Th1 and Th2) [6]. In Th1 responses, interleukin (IL)-2 and interferon (IFN)-γ are secreted, with preferential promotion of cell-mediated immunity, as

well as IgM and IgG2a production by B-cells. In Th2 responses, IL-4 and IL-5 are secreted, with preferential promotion of humoral immunity, including IgE and IgG1 production. In Th2, IL-10 is also secreted, and this can suppress Th1 activity. In contrast to the acute allograft rejection that is mediated mainly by Th1 cellular responses, CRAI is reportedly mediated by Th2 humoral responses, especially anti-HLA antibodies produced after transplantation [7-12]. In some cases, a Th population, Th3, regulates both Th1 and Th2 activity by secreting an immunosuppressive cytokine, transforming growth factor (TGF)-β1, which is a potent inducer of regulatory T cells (Tregs) [13-17]. TGF-β1 is also known to protect kidneys from ischemia-reperfusion injury in transplantation [18-21]. On the other hand, TGF-β1 promotes glomerular fibrogenesis, vasculitis, and cyclosporin A-induced toxicity in allografts, all of which are associated closely with the pathogenesis of CRAI [22-27]. Thus TGF-β1 has been reported to serve as both a protective and CRAI-causing cytokine in kidney transplantation [28].

In our previous analysis of renal allograft-infiltrating cells using biopsy specimens, we found that CRAI was mediated by both $CD4^+$ and $CD8^+$ cells, and that the Th1-type cytokine IFN-γ was closely related to CRAI [29]. In the present study, to assess the systemic immune status during progression of CRAI, we analyzed the plasma of patients who had undergone kidney transplantation, focusing on the levels of Th1 and Th2 cytokines as well as TGF-β1 and the appearance of anti-HLA class I and class II antibodies of both the IgG and IgM immunoglobulin classes.

Materials and Methods

Patients

All patients analyzed in this study underwent kidney transplantation at Kitasato University Hospital (57 men and 30 women; 59 living donors and 28 cadaveric donors) (Table 1). This study was performed with the informed consent of all patients and was approved by the ethics committee of Kitasato University School of Medicine. CRAI was diagnosed pathologically according to the Banff '07 classifications [30] for the specimens taken at the periodical biopsy. Post-transplantation periods at the time of pathological examination and analysis of the plasma creatinine, cytokines, and antibodies are shown in Table 2.

Plasma cytokines and antibodies

The plasmas were collected from the patients at the time of periodical medical examination and concentrations of IL-2, IFN-γ, IL-4, IL-10, and TGF-β1 were measured using commercial ELISA kits (Biosource Inc.). Anti-HLA class I and class II antibodies were detected by reacting the plasma with FlowPRA class I and class II beads, respectively (HLA antibody screening beads, One Lamda Inc.). Antibodies of the IgG and IgM immunoglobulin classes were detected by flowcytometry using fluorescein isothiocyanate -conjugated secondary antibodies against each human immunoglobulin class. Statistical comparisons were performed using ANOVA, chi-squared test, and student's t-test.

Table 1. Classification of patients analyzed in this study.

			donor		plasma creatinine (av±sd)
			living	cadaver	(mg/dl)
Normal creatinine group	men	15	8	7	0.5-1.2 (1.0±0.2)
	women	5	2	3	0.5-0.8 (0.6±0.1)
High creatinine group	men	42	31	11	1.3-7.4 (2.2±1.3)
	women	25	18	7	0.9-5.4 (1.8±1.1)

Table 2. Post-transplantation periods at the time of pathological examination and the analysis of plasma creatinine level.

without CRAI (n=37)		CRAI (n=40)	
post-transplantation period	creatinine (mg/dl)	post-transplantation period	creatinine (mg/dl)
0y 6m	0.4	0y 6m	4.4
1y 5m	1.2	0y 9m	3.7
2y 3m	1.0	1y 0m	1.7
2y 3m	1.8	1y 10m	1.1
2y 10m	1.1	1y 6m	1.2
2y 11m	1.0	1y 6m	1.4
3y 0m	1.4	1y 8m	1.9
4y 2m	1.5	2y 3m	1.7
5y 9m	1.0	2y 9m	0.6
5y 11m	1.6	3y 0m	2.3
6y 3m	1.1	3y 3m	2.4
7y 1m	0.9	3y 5m	2.0
8y 1m	1.3	3y 8m	4.0
9y 2m	1.9	3y 9m	2.6
9y 11m	1.3	3y 10m	2.3
10y 5m	0.6	6y 4m	1.8
10y 10m	1.3	8y 0m	1.3
11y 5m	1.6	8y 5m	1.7
11y 8m	0.5	9y 8m	1.5
12y 6m	0.8	9y 9m	1.4

13y 5m	1.4	9y 10m	2.7
14y 0m	1.5	11y 2m	2.2
14y 9m	1.4	13y 0m	2.4
15y 0m	1.1	13y 1m	1.6
15y 6m	1.1	13y 2m	2.0
15y 7m	1.1	14y 0m	2.4
15y 8m	0.7	14y 0m	2.5
16y 6m	1.7	14y 10m	3.7
17y 5m	1.0	15y 3m	2.3
18y 1m	1.5	15y 7m	1.8
23y 0m	1.0	15y 8m	7.4
23y 8m	1.1	16y 0m	1.5
24y 4m	1.3	16y 2m	5.4
25y 8m	1.0	16y 3m	2.1
27y 6m	1.7	16y 9m	2.0
28y 0m	1.2	16y 10m	3.0
29y 5m	0.5	16y 11m	6.2
		18y 3m	1.2
		18y 9m	1.3
		27y 6m	4.9

Results

Comparison of plasma cytokine levels between patients with normal and high creatinine levels

First, 87 patients who had undergone kidney transplantation were divided into two groups, i.e., a normal creatinine group (n=20) and a high creatinine group (n=67), using plasma creatinine cut-off levels of 1.3 mg/dl (for men) and 0.9 mg/dl (for women) (Table 1). Analysis of Th1-type cytokines (IL-2 and IFN-γ and Th2-type cytokines (IL-4 and IL-10) in the plasma of patients indicated no significant difference in the levels of any of these cytokines between the normal and high creatinine groups (Table 3).

Table 3. Comparison of plasma cytokine concentrations between patients with normal and high level of plasma creatinine.

Cytokine	Normal creatinine group (n=20)	High creatinine group (n=67)	p value in ANOVA
IL-2 (U/ml)	0.93±0.62	1.11±0.63	0.26
IFN-γ (pg/ml)	4.44±4.75	5.73±4.37	0.26
IL-4 (pg/ml)	0.83±3.17	0.43±1.95	0.50
IL-10 (pg/ml)	1.23±1.50	0.92±1.54	0.44
TGF-β1 (ng/ml)	82.5±30.5	66.3±29.2	0.036*

On the other hand, the plasma level of the immunosuppressive cytokine TGF-β1 was significantly higher in the normal creatinine group than in the high creatinine group (p=0.036). This result indicated that the higher plasma TGF-β1 level was associated with functional preservation of kidney allografts.

Comparison of plasma cytokine levels between patients without CRAI and patients with CRAI

Next, patients were divided into two groups based on the pathological observation, i.e., those without CRAI and those with CRAI. The group of patients with CRAI (n=40) showed significantly higher plasma creatinine concentration than the group of patients who did not show CRAI (n=37) (Figure1, $p=5.7x10^{-6}$), and the episode of CRAI correlated well with the above-defined classification of the normal or high creatinine level (Table 4, p=0.0003).

Figure 1. Plasma creatinine concentrations of patients without CRAI and patients with CRAI. The average plasma creatinine concentration in the group of patients without CRAI (n=37) and that in the group of patients with CRAI (n=40) were 1.20±0.37 and 2.43±1.48 mg/dl, respectively; p value by student's t-test $=5.7x10^{-6}$.

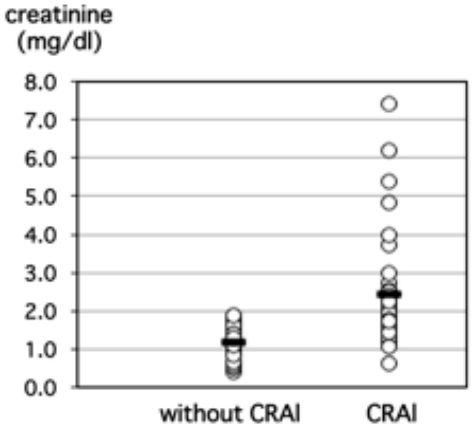

Table 4. Interrelationship between the plasma creatinine level and episode of CRAI.

Plasma creatinine level	without CRAI	CRAI
Normal	16	3
High	21	37

p value in chi-square test: 0.0003

The levels of plasma cytokines were compared between the group without CRAI and the group with CRAI (Table 5). The levels of IL-2, IFN-γ, IL-4, and IL-10 did not differ between the two groups, indicating that neither plasma Th1 nor Th2 cytokines were associated with CRAI. The plasma level of TGF-β1, on the other hand, was significantly higher in the group without CRAI than in the group with CRAI (p=0.011), suggesting that the higher plasma TGF-β1 level was associated with prevention of CRAI.

Table 5. Comparison of plasma cytokine concentrations between patients with and without an episode of CRAI.

Cytokine	without CRAI (n=37)	CRAI (n=40)	p value in chi-square test
IL-2 (U/ml)	0.95±0.70	1.08±0.57	0.35
IFN-γ (pg/ml)	5.42±5.18	5.00±3.60	0.33
IL-4 (pg/ml)	0.55±2.29	0.12±0.26	0.25
IL-10 (pg/ml)	0.90±1.29	1.03±1.11	0.64
TGF-β1 (ng/ml)	80.0±29.6	62.0±30.2	0.011*

Appearance of anti-HLA antibodies in patients' plasma

Finally, we analyzed the appearance of anti-HLA class I and class II antibodies of both IgG and IgM immunoglobulin classes in the plasma of patients. It was found that IgM antibodies against HLA class I and/or class II antigens were present at a significantly higher frequency in the high creatinine group than in the normal creatinine group, suggesting a possible association of anti-HLA IgM antibodies with functional impairment of allografts (Table 6). Comparisons of other anti-HLA antibodies between the group without CRAI and the group with CRAI revealed no statistically significant differences (Table 7).

Table 6. Frequency of anti-HLA antibody in patients with normal and high level of plasma creatinine.

Antibody		Number of patients with antibody: Normal creatinine group (n=20)	High creatinine group (n=67)	p value in chi-square test
HLA-class I	IgG	2	6	0.89
HLA-class I	IgM	1	14	0.10
HLA-class I	IgG and/or IgM	3	18	0.28
HLA-class II	IgG	2	8	0.81
HLA-class II	IgM	0	7	0.13
HLA-class II	IgG and/or IgM	2	12	0.40
IgG	HLA-class I and/or II	4	13	0.95
IgM	HLA-class I and/or II	1	19	0.029*
Any of HLA antibodies		4	25	0.14

Table 7. Frequency of anti-HLA antibody in patients with and without an episode of CRAI.

Antibody		Number of patients with antibody: without CRAI (n=37)	CRAI (n=40)	p value in chi-square test
HLA-class I	IgG	2	5	0.28
HLA-class I	IgM	7	9	0.70
HLA-class I	IgG and/or IgM	9	13	0.43
HLA-class II	IgG	3	7	0.22
HLA-class II	IgM	3	4	0.77
HLA-class II	IgG and/or IgM	5	9	0.31
IgG	HLA-class I and/or II	5	11	0.13
IgM	HLA-class I and/or II	9	12	0.58
Any of HLA antibodies		13	18	0.38

Discussion

Although numerous studies have been reported, the mechanism responsible for CRAI has not yet been conclusively established [1]. Previously, we analyzed cytokine mRNAs expressed by renal allograft-infiltrating cells in biopsy specimens to clarify the type of helper T cell, Th1 or Th2, playing the more important role in the progression of CRAI [29]. We found that the Th1-type cytokine IFN-γ was closely associated with CRAI, suggesting that mild but recurrent cell-mediated immune reaction may progress to CRAI. However, in the present analysis of plasma cytokines to assess the systemic immune status of patients, we did not observe any significant elevation of IFN-γ. It is possible that the level of IFN-γ produced locally in allografts may not have been high enough to be detected as a significant increase in plasma.

The present analysis revealed that the level of plasma TGF-β1 was significantly higher in patients with normal levels of creatinine than in those with high levels, and also significantly higher in patients without CRAI than in those with CRAI. Because TGF-β1 is known to be a potent inducer of Tregs, which could effectively suppress allogeneic immune reactions [13-17], the higher plasma level of TGF-β1 may represent a systemic immune status that is favorable for preventing recurrent cell-mediated immune reaction leading to CRAI [31]. Also, if patients had a high level of TGF-β1 at the time of transplantation, although not apparent in this study, this cytokine might have ameliorated the degree of ischemia-reperfusion injury to allografts after surgery [18-21]. On the other hand, TGF-β1 has also been reported to exacerbate CRAI pathology in allografts

in the form of fibrogenesis, vasculitis, and cyclosporin A-induced toxicity [22-27]. Further investigations will be needed to clarify how the balance between a protective or harmful effect of TGF-β1 is determined, and from a clinical viewpoint, how to manipulate its effect toward a protective role.

Anti-HLA antibodies, in particular those of the IgG class, are known to be closely related to the progression of CRAI [7-12]. The deposition of complement products in allografts also indicates the importance of the humoral immune reaction [9-11]. Although IgM-class antibodies have been regarded as less harmful, several recent studies have demonstrated their significance and relevance in CRAI [7,32-34]. In the present study, we also observed a higher frequency of anti-HLA IgM antibody in the group of patients with impaired kidney function. Because IgM is produced from the beginning of the primary immune response and is persistently present in the later stage, and because it is an efficient activator of the complement system, it is possible that anti-HLA IgM antibody would have greater significance than considered previously in the progression of kidney allograft failure.

Conclusions

Analyses of plasma cytokines of patients who received kidney allografts indicated that transforming growth factor-β1 would be helpful to preserve the function of kidney allografts by suppressing the progression of chronic renal allograft injury. Analyses of anti-HLA antibodies suggested that anti-HLA IgM antibodies against HLA class I and/or class II may be implicated in functional impairment of kidney allografts.

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