Positive Flow Cytometry Crossmatch Reactivity with Pronase-Treated T-Cells Induced by Non-HLA Auto-Reactive Antibodies in Human Immunodeficiency Virus-Infected Patients

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Introduction

Historically, a positive flow cytometry crossmatch (FCXM) has been considered a contraindication for transplantation. Although interpreting the results of the T-cell FCXM has had few problems, the B-cell FCXM has been problematic due to the presence of Fc receptors on the cell membrane. IgG can bind to Fc receptors, resulting in increased background fluorescence, affecting the sensitivity and specificity. Pronase treatment of lymphocytes was subsequently introduced to improve the sensitivity and specificity of the B-cell FCXM. However, pronase treatment of both T- and B-cells in a single tube has been widely adopted for the 3-color FCXM. Although pronase treatment has been shown to increase the sensitivity and specificity of the B-cell FCXM, its effects on the T-cell FCXM have not been well documented. In this regard, we have observed a significantly high rate of positive T-cell FCXM results in the absence of anti-HLA donor-specific antibodies (DSA) in patients infected with the human immunodeficiency virus (HIV). This study was aimed at determining the cause of these aberrant results.

Materials & Methods

Patients: Twenty-six HIV+ and 30 HIV- patients with similar demographics were included in this study (Table 1). A total of 348 sera from HIV+ patients were tested with pronase-treated (PT) cells, and 81 sera were tested with non-treated (NT) cells from 196 different deceased donors. A total of 60 sera from HIV- patients were tested with PT and NT cells from 48 different deceased donors.

Cell Isolation: Peripheral blood or lymph node lymphocytes, CD4+ T-cells and CD8+ T-cells were isolated by means of negative selection using the RoboSep Automated Cell Separator (StemCell Technologies).

Pronase Treatment: Cells were incubated at 37ºC for 15 min. with pronase (1 mg/ml, Sigma). The cells were then washed with RPMI-1640 containing 30% fetal bovine serum (FBS) and then washed with RPMI-1640 containing 10% FBS. Of note, pronase cleaves both CD4 and CD8 markers from the T-cell membrane.

Serum Pre-Treatment: HIV+ sera (PRA=0%) were pre-treated with various reducing agents: Dithiothreitol (DTT), 2-Mercaptoethanol (2-ME) and Tris [2-Carboxyethyl] Phosphine (TCEP) (Sigma-Aldrich) and then crossmatched with PT and NT T-cells.

Serum Pre-Absorption: HIV+ sera (PRA=0%) (120 µl) were pre-absorbed with PT and NT T-cells ($10x10^6$) and PT and NT B-cells ($10x10^6$) for 60 minutes at 25ºC and then crossmatched with allogeneic PT T-cells.

FCXM: Cells ($3x10^5$) were incubated with serum (50 µl) for 30 min. at 25ºC. Then, FITC-conjugated $F(ab')_2$ goat anti-human IgG (Fc-specific) (Jackson ImmunoResearch Labs) was added and incubated for 20 min. at 25ºC. T- and B-cells were analyzed using PerCP-conjugated anti-CD3 and PE-conjugated anti-CD19 mAbs (BD Biosciences), respectively, in a BD FACSCalibur instrument (BD Biosciences). Fluorescence intensity was acquired as logarithmic data, and the difference between the median fluorescence for each test serum and the negative control serum was calculated and expressed as fluorescence median channel shift (MCS).

Statistic Analysis: Differences in FCXM reactivity (MCS) were assessed by means of the 2-tailed Student's t-test using the Prism 5.0 program (GraphPad Software) with the α set at $p<0.01$.

Results

Table 1

Patient Demographics

	HIV+	HIV-
N	26	30
Males	23 (88.5%)	26 (86.7%)
Females	3 (11.5%)	4 (13.3%)
PRA% Class I	5.1 ± 9.8	3.1 ± 5.4
PRA% Class II	3.1 ± 8.1	0.6 ± 2.1
DSA	-	-

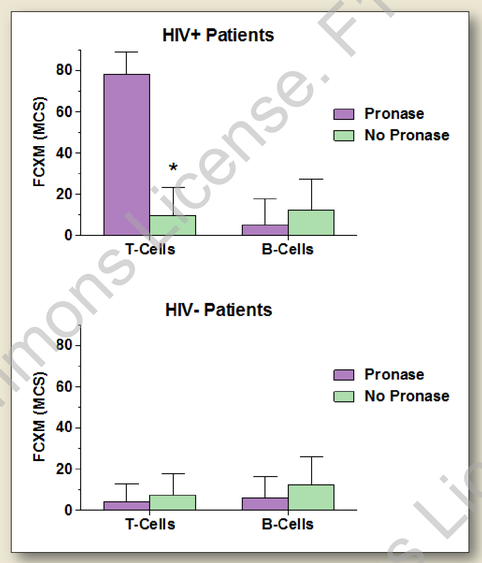

Figure 1. Positive FCXM reactivity against PT T-cells in HIV+ patients without DSA. FCXMs were performed in HIV+ and HIV- patients (PRA=0%) with PT (n=348) and NT (n=81) cells. HIV+ patients exhibited positive T-cell FCXM results with PT cells. In contrast, these patients exhibited negative T-cell FCXMs with NT cells (*$p<0.01$). These patients also exhibited negative B-cell FCXMs with both PT and NT cells. As expected, HIV- patients exhibited negative T- and B-cell FCXMs with both PT and NT cells (n=60). Positive T- and B-cell FCXMs represented MCS≥20 and ≥30, respectively.

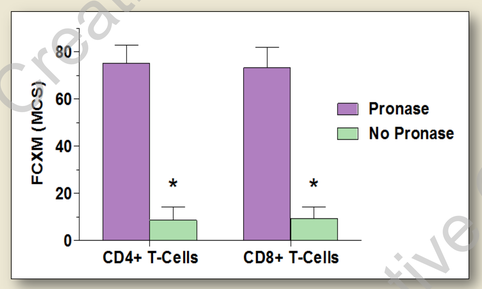

Figure 2. Positive FCXM reactivity with PT CD4+ and CD8+ T-cells in HIV+ patients without DSA. FCXMs were performed in HIV+ patients (PRA=0%) with PT and NT CD4+ and CD8+ T-cells gated with the CD3 marker (n=5). HIV+ patients exhibited positive FCXMs with both PT CD4+ and CD8+ T-cells. In contrast, these patients exhibited negative FCXMs with both NT CD4+ and CD8+ T-cells (*$p<0.01$). Positive T-cell FCXMs represented MCS≥20.

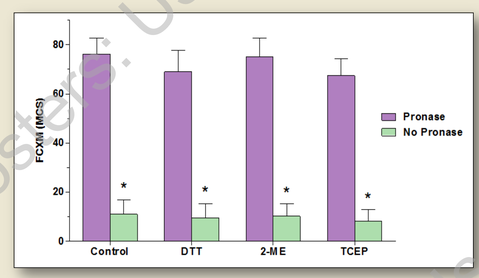

Figure 3. Positive FCXM reactivity against PT T-cells in HIV+ patients without DSA is not reduced by sera pre-treatment with reducing agents. FCXMs were performed in HIV+ patients (PRA=0%) with PT and NT T-cells (n=5). Sera was pre-treated with different reducing agents that have been shown to reduce non-specific antibody binding (DTT, 2-ME and TCEP). Pretreated-sera exhibited positive FCXMs with PT T-cells comparable to that of the control sera. In contrast, these sera exhibited negative FCXMs with NT T-cells (*$p<0.01$). Positive T-cell FCXM results represented MCS≥20.

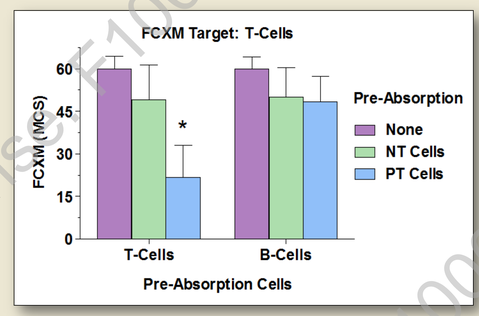

Figure 4. Positive FCXM reactivity against PT T-Cells in HIV+ patients without DSA is reduced by sera pre-absorption with PT T-cells but not with NT T-cells. FCXMs were performed in HIV+ patients (PRA=0%) with PT T-cells (n=5). Sera was pre-absorbed with allogeneic PT and NT T- and B-cells. Pre-absorption of sera with PT T-cells significantly reduces the strength of the FCXM (*$p<0.01$). In contrast, pre-absorption of sera with NT T-cells did not have a significant effect on the strength of the FCXM. In addition, pre-absorption of sera with PT or NT B-cells did not have a significant effect on the strength of the FCXM. Positive T-cell FCXM results represented MCS≥20.

Table 2

Long-Term Kidney Graft Survival Time in HIV+ Patients

Patient #	Graft Survival Time (Days)	T-Cell FCXM (PT)	T-Cell FCXM (NT)
1	2176	Positive	Negative
2	1655	Positive	Negative
3	1637	Positive	Negative
4	1552	Positive	Negative
5	1505	Positive	Negative
6	1412	Positive	Negative
7	1241	Positive	Negative
8	1238	Positive	Negative
9	554	Positive	Negative
10	490	Positive	Negative
11	486	Positive	Negative
12	463	Positive	Negative
MST ± SD	1,201 ± 571		

Discussion

- Pronase treatment induces positive T-cell FCXM in HIV+ patients without DSA. This phenomenon is not observed on B-cells and it is not corrected with pre-treatment with reducing agents. The data indicate that this phenomenon is not due to non-specific antibody binding.
- Reduction of the positive T-cell FCXM reactivity observed on HIV+ sera without DSA by pre-absorption with PT T-cells but not with NT T-cells or PT and NT B-cells indicates that this reactivity is due to specific binding of auto-reactive antibodies to cryptic epitopes exposed by pronase on T-cells.
- A positive T-cell FCXM with PT cells should not be considered a contraindication for transplantation in HIV+ patients.
- Laboratories using pronase should consider testing HIV+ patients with NT cells to prevent these patients from being inappropriately excluded from receiving an organ due to positive T-cell FCXM due to non-deleterious auto-reactive antibodies.

References

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