Human Immunology 77 (2016) 449–455

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Flow cytometry crossmatch reactivity with pronase-treated T cells induced by non-HLA autoantibodies in human immunodeficiency virus-infected patients

Katarzyna Szewczyk [a,b], Kelly Barrios [a], Daniel Magas [a], Kristin Sieg [a], Bozena Labuda [a], Martin D. Jendrisak [a], Andrés Jaramillo [a,b,*]

[a] Gift of Hope Organ & Tissue Donor Network, Itasca, IL 60143, USA
[b] Department of Laboratory Medicine and Pathology, Mayo Clinic Hospital, Phoenix, AZ 85054, USA

ARTICLE INFO

Article history:
Received 16 February 2016
Revised 6 April 2016
Accepted 13 April 2016
Available online 16 April 2016

Keywords:
Flow Cytometry Crossmatch
Pronase
Autoantibodies
Human Immunodeficiency Virus

ABSTRACT

Pronase treatment is used in the flow cytometry crossmatch (FCXM) to prevent nonspecific antibody binding on B cells. However, we have observed unexpected positive results with pronase-treated T cells in human immunodeficiency virus (HIV)-infected patients. In this study, 25 HIV-infected patients without HLA antibodies were tested with pronase-treated and nontreated cells. HIV-positive sera were pretreated with reducing agents and preabsorbed with pronase-treated and nontreated T or B cells before crossmatching. All patients displayed FCXM reactivity with pronase-treated T cells but not with nontreated T cells. None of the patients exhibited FCXM reactivity with pronase-treated and nontreated B cells. These patients displayed FCXM reactivity with pronase-treated CD4+ and CD8+ T cells but not with their nontreated counterparts. Preabsorption with pronase-treated T cells reduced the T cell FCXM reactivity. Preabsorption with pronase-treated B cells or nontreated T and B cells did not have any effect on the T cell FCXM reactivity. Pretreatment with reducing agents did not affect the T cell FCXM reactivity. 15 of 21 HIV-infected kidney allograft recipients with pronase-treated T cell FCXM reactivity display long-term graft survival (1193 ± 631 days). These data indicate that HIV-infected patients have nondeleterious autoantibodies recognizing cryptic epitopes exposed by pronase on T cells.

1. Introduction

Highly active antiretroviral therapy has dramatically improved the life expectancy in human immunodeficiency virus (HIV)-infected patients. However, multiple organ failure has emerged in many of these patients who are now living longer. In 2013, the HIV Organ Policy Equity Act which would allow HIV-infected patients to donate their organs to other HIV-infected patients was placed into effect [1–4]. On May 8, 2015, the U.S. Department of Health and Human Services announced that it will amend the Organ Procurement Transplantation Network Final Rule (42 CFR Part 121) to allow the recovery of transplantable organs from HIV-infected donors [1–4]. The Organ Procurement Transplantation Network fully supports the HIV Organ Policy Equity Act in the interest of increasing the number of transplants, a primary strategic goal for the national transplant network. This will increase the number of HIV-infected patients who will be tested and the number of HIV-infected patients receiving organ allografts [1–4]. Previously no organs from HIV-infected donors could be used for transplant purposes, despite the fact that a considerable number of candidates on the transplant waiting lists are HIV-infected. In this regard, the National Institute of Allergy and Infectious Diseases is leading the development of the criteria for transplant programs that plan to accept HIV-positive organs for HIV-infected recipients. These measures will provide the framework for studies to begin as early as 2016 [1–4].

The flow cytometry crossmatch (FCXM) is the most sensitive cell-based method for detecting HLA antibodies [5–10]. Thus, a

Abbreviations: DTT, dithiothreitol; FCXM, flow cytometry crossmatch; FITC, fluorescein isothiocyanate; HIV, human immunodeficiency virus; MCS, median channel shift; mAb, monoclonal antibody; PRA, panel reactive antibody; SD, standard deviation; TCEP, tris(2-carboxyethyl)phosphine; 2-ME, 2-mercaptoethanol.

* Corresponding author at: Department of Laboratory Medicine and Pathology, Mayo Clinic Hospital, 5777 E. Mayo Blvd., Phoenix, AZ 85054, USA.
E-mail address: jaramillo.andres@mayo.edu (A. Jaramillo).

http://dx.doi.org/10.1016/j.humimm.2016.04.014
0198-8859/

positive FCXM result is usually considered a contraindication for transplantation [5–10]. However, the presence of Fc receptors has made the B cell FCXM more problematic in interpretation of results [11–16]. Nonspecific binding of IgG antibodies to Fc receptors on B cells decreases the sensitivity and specificity of the B cell crossmatch [11–16]. Pronase treatment of lymphocytes was subsequently introduced to improve the sensitivity and specificity of the B cell FCXM to remove Fc receptors from the cell membrane [11–16]. Pronase treatment of unseparated T and B cells has been widely adopted for the 3-color FCXM [11–16]. Several studies have demonstrated the improved sensitivity and specificity of the B cell FCXM in detecting HLA antibodies with the use of pronase [11–16].

In our laboratory, we have observed a significantly high rate of positive T cell FCXM results in HIV-infected patients in the absence of donor-specific antibodies. The data presented herein indicate that this positive FCXM reactivity is due to autoantibodies recognizing cryptic epitopes exposed by pronase treatment on T cells. In this regard, previous studies have shown that the majority (94%) of HIV-infected patients have autoantibodies directed against T cells [17–21].

2. Materials and methods

2.1. Patients

HIV-infected and HIV-negative patients without HLA antibodies (panel reactive antibody, PRA = 0%) and with similar demographics were included in this study (Table 1). A total of 348 serum samples from HIV-infected patients were tested with pronase-treated cells (n = 196), and 81 of these serum samples were tested with nontreated cells (n = 48) from different deceased donors. A total of 60 serum samples from HIV-negative patients were tested with pronase-treated and nontreated cells (n = 48) from different deceased donors.

2.2. HLA antibody analysis

The presence of PRA was assessed by means of the LABScreen PRA Class I and Class II Luminex assays (One Lambda, Inc.) as well as the LABScreen Single Antigen HLA Class I and Class II Luminex assays (One Lambda, Inc.). Positive results for the single antigen bead assays represented mean fluorescence intensity values greater than 700.

2.3. Lymphocyte isolation

Whole lymphocytes, CD4+ T cells, and CD8+ T cells were isolated from peripheral blood or lymph nodes by means of negative selection using the EasySep HLA Total Lymphocyte Enrichment kit, the EasySep Human CD4+ T Cell Isolation kit, and the EasySep Human CD8+ T Cell Isolation kit, respectively (STEMCELL Technologies) with the RoboSep automated cell separator (STEMCELL Technologies).

Table 1
Patient demographics.

	HIV-Infected (n = 25)	HIV-Negative (n = 30)
Males	22 (88%)	26 (86.7%)
Females	3 (12%)	4 (13.3%)
PRA Class I[a]	0%	0%
PRA Class II[a]	0%	0%

Abbreviations: HIV, human immunodeficiency virus; PRA, panel-reactive antibody.
[a] The presence of PRA was assessed by means of the LABScreen PRA Class I and Class II Luminex assays (One Lambda, Inc.) as well as the LABScreen Single Antigen HLA Class I and Class II Luminex assays (One Lambda, Inc.).

2.4. Pronase treatment

Pronase treatment was performed by an adaptation of previously described methods [11–16]. Briefly, 10×10^6 whole lymphocytes, CD4+ T cells, or CD8+ T cells were incubated at 37 °C for 15 min in the presence of pronase (1 mg/mL) (type XIV, Sigma–Aldrich). The cells were then washed twice with RPMI-1640 medium containing 30% fetal bovine serum and resuspended in RPMI-1640 medium containing 10% fetal bovine serum before use. To determine the effect of the pronase treatment, CD20 expression was assessed using a fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD20 monoclonal antibody (mAb) (clone 2H7; BD Biosciences). Of note, pronase treatment at a concentration of 1 mg/mL does not completely remove CD20 from the cell membrane. Lymphocytes need to be treated with a higher concentration of pronase (2 mg/mL) to completely remove membrane-bound CD20 [11,22]. However, this higher concentration of pronase induces a considerably high percentage of cell death with final cell yields of 25–50% of the original cell number [11]. Thus, pronase treatment at 1 mg/mL was considered acceptable when CD20 expression was 25% or lower than the one observed on nontreated cells (data not shown) [22].

2.5. Serum pretreatment with reducing agents

Selected sera from HIV-infected patients were pretreated with dithiothreitol (DTT, 5 mM) (Sigma–Aldrich), 2-mercaptoethanol (2-ME, 10 mM) (Sigma–Aldrich), or tris(2-carboxyethyl) phosphine (TCEP, 2 mM) (Sigma–Aldrich) for 45 min at 37 °C and then tested with pronase-treated and nontreated T cells.

2.6. Serum preabsorption

Selected sera from HIV-infected patients (120 μL) were preabsorbed with pronase-treated and nontreated T and B cells (10×10^6) for 60 min at 25 °C and then tested with syngeneic pronase-treated T cells.

2.7. Flow cytometry crossmatch

Lymphocytes (3×10^5) were incubated with test or control sera (50 μL) for 30 min at 25 °C, followed by 3 washes with wash buffer (phosphate-buffered saline solution containing 5% heat-inactivated fetal bovine serum and 0.1% NaN_3). Then, an optimized concentration of a FITC–conjugated $F(ab')_2$ goat anti-human IgG (Fc-specific) antibody (Jackson ImmunoResearch Laboratories, Inc.) was added and incubated for 20 min at 25 °C, followed by 2 washes with wash buffer. T and B cells were analyzed using peridinin-chlorophyll-protein complex-conjugated mouse anti-human CD3 (clone SK7; BD Biosciences) and phycoerythrin-conjugated anti-human CD19 (clone SJ25C1; BD Biosciences) mAbs, respectively, on a FACSCalibur flow cytometer (BD Biosciences). Fluorescence intensity was acquired as logarithmic data and the difference between the fluorescence median channel for each test serum and the negative control serum was calculated and expressed as fluorescence median channel shift (MCS) values. A qualitative assessment of HLA expression was determined by staining cells with FITC-conjugated mouse anti-HLA class I (HLA-A, B, C; clone W6/32; Sigma–Aldrich) and anti-HLA class II (HLA-DR, DQ, DP; clone Tu39; BD Biosciences) mAbs. For both pronase-treated and nontreated T cell FCXM reactivity, positive results represented MCS values greater than 20. In addition, for pronase-treated and nontreated B cell FCXM reactivity, positive results represented MCS values greater than 30.