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The Single Antigen expressing Lines (SALs) Concept: An Excellent Tool for Screening for HLA-Specific Antibodies

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ABSTRACT: Definition of the antibody specificity in the serum of patients waiting for a renal transplant or in need for platelet transfusion is a crucial step for finding adequate donors. Confounding factors are the complexity of the serum antibodies and the expression of several, up to six, different human leukocyte antigens (HLA) on peripheral blood lymphocytes used as target cells in the antibody screening. Single antigen-expressing (SAL) cell lines were generated by transfecting human major histocompatibility complex (MHC) class I sequences into K562, an erythroleukemia-derived cell line lacking MHC class I and II expression. Thirty-seven different SALs have been generated so far. In this study, we present the validation of 16 of those SALs by flow cytometry against a panel of 84 human HLA-specific monoclonal antibodies (30 HLA-A [8 IgG/22 IgM], 45 HLA-B [18 IgG/27 IgM], 6 HLA-A, B [3 IgG/3 IgM], and 3 HLA-C [all IgM]) developed in our laboratory. The SALs proved to be suitable tools to determine acceptable mismatches for highly sensitized patients. This concept of transfecting target sequences in immortalized cell lines opens up new avenues in the definition of serum and cellular reactivity for sensitized patients awaiting a suitable organ or blood component. *Human Immunology 66, 519–525 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: Highly sensitized; HLA antibodies; kidney transplantation; K562; screening

ABBREVIATIONS

AM	acceptable mismatch
CDC	complement-dependent cytotoxicity
FCM	flow cytometry
ELISA	enzyme-linked immunosorbent assay
HLA	human leukocyte antigen
HuMAb	human monoclonal antibody
Ig	immunoglobulin
MHC	major histocompatibility complex
PBL	peripheral blood lymphocyte
PRA	panel-reactive antibody
SAL	single antigen line

INTRODUCTION

One of the most important tasks of laboratories involved in solid-organ transplantation and blood transfusion is the definition of the specificity of the sensitization toward human leukocyte antigens (HLAs). Donor-specific HLA antibodies may cause hyperacute graft rejection [1, 2]; platelet transfusions in the presence of antidonor HLA antibodies are associated with platelet refractoriness [3]; and HLA antibodies have been correlated with transfusion-related acute lung injury [4]. Therefore, screening patients' sera for HLA-specific antibodies is essential. Human leukocyte antigen alloantibody screening is pri-

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Supported by the Dutch Kidney Foundation (Nierstichting); grant number C10.1920 (WC20).

Received September 11, 2004; revised January 6, 2005; accepted January 7, 2005.

0198-8859/05/$–see front matter
doi:10.1016/j.humimm.2005.01.007

marily based on the complement-dependent cytotoxicity (CDC) assay, the flow cytometry (FCM) assay [5–7], and, recently, enzyme-linked immunosorbent assays (ELISA) [8]. Large panels of HLA-typed cells are used to determine the antibody specificity. These cells are usually heterozygous for the antigens HLA-A, HLA-B, and HLA-C and therefore express up to six different antigens, which makes the definition of serum antibodies in highly sensitized patients a complex task. The antigen preparations for these assays were either recombinant [8, 9] or isolated from platelets, cell lines, or their supernatants. The antigens were then immobilized on polystyrene supports (beads, microwell plates). Any of these conditions might result in subtle changes in the tertiary structure of the antigens [9]. Here, we present a concept that avoids the problem of complexity and at the same time provides the major histocompatibility complex (MHC) class I antigen in a cell surface–bound form, enabling a definition of HLA antibody specificities that is simple, more reliable, and less costly. Using K562 [10], an erythroleukemia cell line, which lacks expression of all MHC class I and II antigens, as a recipient for separate transfection of MHC encoded class I antigens, we generated cell lines with natural cell surface expression that are suitable targets for the antibody assays. Validation of this concept was performed using human monoclonal HLA antibodies (HuMAbs [11]) of well-defined specificity as models for complex sera. Furthermore, the single antigen lines (SALs) were shown to be useful for the detection of acceptable HLA mismatches for highly sensitized patients.

MATERIALS AND METHODS

Generation of Transfectants

Plasmid constructs (pLNCX, ampicillin and neomycin resistant) containing various MHC class I heavy chain genes (Table 1) were obtained from the 13th International Histocompatibility Working Group and were transfected in K562 cells [12], obtained from the American Type Culture Collection (Manassas, VA, order number CLL-243) [10] by electroporation using the Genepulser (Bio-Rad, Hercules, CA) with instrument settings of 230 V and 960 μF. Electroporation was performed with 10^7 cells and 10 μg of plasmid DNA. On day 2 after transfection, selection was started with G418 (neomycin derivative, final concentration: 200 μg/ml; Invitrogen, Groningen, The Netherlands). The antibiotic-resistant transfectants were expanded for at least 2 weeks. Major histocompatibility complex class I–positive cells were enriched by cell sorting using w6/32 coated antimouse immunoglobulin (Ig) magnetic beads (Dynal, Oslo, Norway). The mouse MAb w6/32 recognizes a monomorphic conformational MHC class I epitope [13]. Sorted cells were expanded using G418, tested for class I expression with w6/32, and cryopreserved in multiple aliquots.

TABLE 1 Currently available panel of single antigen lines

A*0101#	B*0702#	Cw*0102
A*0201#	B*1301	Cw*0303
A*0301 (2x)#	B*1402	Cw*0304
A*1101#	B*1501	Cw*0401
A*2402	B*2705	Cw*0602
A*2601	B*3501	Cw*0801
A*3101	B*3801	Cw*1202
A*3201	B*3901	Cw*1402
A*3303	B*4001#	Cw*1502
A*6901	B*4002	
	B*4402	
	B*4403#	
	B*4501	
	B*4601	
	B*4901	
	B*5101	
	B*5501	

The single antigen lines validated in the present study are underlined.
Abbreviation: # = retroviral vector.

HuMAbs

The HuMAbs used in this study ($n = 84$) were derived from 36 multiparous females by Epstein-Barr Virus (EBV) transformation and electrofusion [14]. Their specificities were defined using large panels ($n > 240$) of HLA-typed mononuclear cells by the CDC assay [5, 11], commercial solid phase assays, and FCM.

Human Sera Selection and Procedure for Acceptable Mismatch Identification

HLA-A2– or HLA-B7–specific sera from multiparous women ($n = 28$ sera; 28 women) were screened using SAL-A2 and SAL-B7 and a panel of HLA-typed peripheral blood lymphocytes (PBLs) from unrelated donors. Sera of highly sensitized patients ($n = 17$ sera; 13 patients) were screened against the panel of the 16 SALs validated in the present report. Acceptable mismatches (AMs) were already defined using selected donors [15]. Acceptable mismatches are HLA mismatches against which the patient never has formed antibodies [15–18]. These antigens are defined in extensive screenings and crossmatches using PBLs from blood donors with a single HLA mismatch to the patient [17].

FCM Assays

Expression. The expression of MHC class I on the surface of SALs was analyzed by incubation of 350,000 cells in a total volume of 50 μl with w6/32 for 30 minutes on ice. After washing with phosphate-buffered saline + 0.1% bovine serum albumin, the cells were labeled with a goat antimouse IgG fluorescein isothiocyanate as secondary