The non-palindromic adaptor-PCR method for the identification of the T-cell receptor genes of an interferon-γ-secreting T-cell hybridoma specific for *trans*-sialidase, an immunodominant *Trypanosoma cruzi* antigen

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Abstract

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Cloning of the T-cell receptor genes is a critical step when generating T-cell receptor transgenic mice. Because T-cell receptor molecules are clonotypical, isolation of their genes requires reverse transcriptase-assisted PCR using primers specific for each different Vα or Vß genes or by the screening of cDNA libraries generated from RNA obtained from each individual T-cell clone. Although feasible, these approaches are laborious and costly. The aim of the present study was to test the application of the non-palindromic adaptor-PCR method as an alternative to isolate the genes encoding the T-cell receptor of an antigen-specific T-cell hybridoma. For this purpose, we established hybridomas specific for *trans*-sialidase, an immunodominant *Trypanosoma cruzi* antigen. These T-cell hybridomas were characterized with regard to their ability to secrete interferon-γ, IL-4, and IL-10 after stimulation with the antigen. A CD3+, CD4+, CD8– interferon-γ-producing hybridoma was selected for the identification of the variable regions of the T-cell receptor by the non-palindromic adaptor-PCR method. Using this methodology, we were able to rapidly and efficiently determine the variable regions of both T-cell receptor chains. The results obtained by the non-palindromic adaptor-PCR method were confirmed by the isolation and sequencing of the complete cDNA genes and by the recognition with a specific antibody against the T-cell receptor variable ß chain. We conclude that the non-palindromic adaptor-PCR method can be a valuable tool for the identification of the T-cell receptor transcripts of T-cell hybridomas and may facilitate the generation of T-cell receptor transgenic mice.

Introduction

Transgenic mice expressing specific T-cell receptors (TCR) are a valuable tool for the study of the immune responses to auto-antigens, infectious agents and neoplastic cells (for reviews, see Refs. 1-6). A critical step when generating these transgenic mice is the isolation of the TCR genes. Because TCR molecules are clonotypical, isolation of these genes requires the identification of the individual TCR expressed by a T-cell clone or hybridoma. This can be accomplished using a panel of TCR-specific monoclonal antibodies. Although monoclonal antibodies are available for the identification of the different Vß chains of the mouse TCR, antibodies specific for many of the murine Vα chains do not exist. Alternatively, cloning can be accomplished by reverse transcriptase-assisted PCR using primers specific for each different Vα or Vß genes or by the screening of cDNA libraries generated from RNA obtained from each individual T-cell clone. Although feasible, these approaches are laborious and costly.

In 1992, Chen and Platsoucas (7) developed the non-palindromic PCR method for the amplification of transcripts with variable or unknown 5' ends, such as TCR and immunoglobulins. Among the advantages of this methodology is the fact that only one 5' end extension primer is required. Since it was first described, this methodology has been successfully used to clone the genes encoding TCR transcripts of T cells isolated from humans and mice (8-12).

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, afflicts 16 to 18 million people in Latin America (13). The cell-mediated immune response (CMI) participates in multiple aspects of Chagas' disease. During the acute phase of infection both CD4 and CD8 T cells have been described as important mediators of protective immunity. Type 1 cells (Th1 and Tc1) are thought to mediate protective immunity in part by their ability to secrete interferon-γ (IFN-γ) (14). During the chronic phase of the disease, most individuals have a strong CMI to parasite antigens. These T cells may help to maintain the low levels of parasitemia preventing the reactivation of acute infection (15). On the other hand, type 1 CMI may be the cause of heart damage during the chronic phase of infection (16,17).

T. cruzi trypomastigotes express an enzyme denominated *trans*-sialidase (TS) on their surface. This enzyme is required for the parasite to obtain sialic acid from host glycoproteins (18). Serological studies have shown that TS is highly immunogenic during natural human infection with *T. cruzi*. Almost all individuals develop antibodies that recognize an epitope composed of amino acid repetitions located at the carboxy-terminal region of TS (19). Chagasic patients also generate antibodies specific for the N-terminal region of TS that strongly inhibit its enzymatic activity (20-22).

In addition to antibodies, the catalytic domain of TS induces type 1 CMI in chagasic patients (23). The role of CMI specific for the catalytic domain of TS was studied during acute infection in mice. Immunization with DNA vaccines elicited strong type 1 CMI and protective immunity against acute infection in BALB/c or C57BL/6 mice (24-26). These experiments provided strong evidence that CMI to TS plays a protective role during acute *T. cruzi* infection. Nevertheless, the participation of CMI to TS during the chronic phase of infection is still unclear. To study this problem, we will need more powerful experimental models using transgenic CD4 and/or CD8 T cells specific for TS. The first step toward the generation of these transgenic animals is the isolation of TCR genes specific for the antigen. To this end, in the present study we established and characterized T-cell hybridomas specific for TS. Using these T-cell hybridomas, we determined whether the non-palindromic adaptor (NPA)-PCR method would be able to

easily identify the TCR transcripts.

Material and Methods

Animals

Female 5- to 8-week-old BALB/c mice were provided by CEDEME-UNIFESP.

Trans-sialidase recombinant protein production

Recombinant TS was produced in *E. coli* transformed with the plasmid pTScat7 (27). The purity of recombinant TS was determined by sodium dodecyl sulfate 10% polyacrylamide gel electrophoresis. A single band of 70 kDa was visualized in the gel. Protein concentration was estimated by the method of Bradford (BioRad, Hercules, CA, USA).

Immunization of mice and culture of lymph node cells

BALB/c mice were immunized with 25 µg of purified recombinant TS emulsified in complete Freund's adjuvant (Sigma, St. Louis, MO, USA). The emulsion was injected subcutaneously into the two hind footpads. Two weeks later, inguinal and popliteal lymph nodes (LN) were collected. LN cells (3 x 10^6/mL) were then cultured in RPMI medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 mM HEPES, 2 g/L sodium bicarbonate, 2 mM L-glutamine, 50 µM 2-mercaptoethanol, 1 mM sodium pyruvate, 1% (v/v) of the nonessential amino acid solution, 1% (v/v) MEM vitamin solution (all purchased from Invitrogen), 100 U penicillin and streptomycin per mL (Sigma), 10 µg/mL recombinant TS, 20 units/mL recombinant human IL-2, and 2% (v/v) inactivated normal human serum. The cultures were maintained for 5 days at 37ºC in an atmosphere containing 5% CO_2 and then harvested and used for fusion.

Fusion and screening of the T-cell hybridomas

Specific LN cells and BWα-ß-thymoma cells (kindly provided by Dr. P. Marrack, University of Colorado Health Science Center, CO, USA) were mixed at a 5:1 ratio. One milliliter FCS-free MEM (Invitrogen) containing 50% polyethylene glycol 1450 solution (w/v, Sigma) was added to the cell pellet under continuous gentle shaking at 37ºC. After 1 min at 37ºC, 50 mL MEM was slowly added. Cells were centrifuged at 250 *g* for 6 min. The cells were resuspended in MEM supplemented with 2.86 g/L sodium bicarbonate, 0.6 g/L D-dextrose, 2 mM L-glutamine, 30 µM 2-mercaptoethanol, 1 mM sodium pyruvate, 1.1% (v/v) of the nonessential amino acid solution, 0.3% (v/v) essential amino acid solution, 80 U/mL penicillin G, 60 U/mL streptomycin sulfate (10% S-MEM), and 10% FCS (HyClone, Logan, UT, USA). Serial dilutions were made with this cell suspension. Cloning was achieved by limiting dilution. One hundred microliters of the suspension was poured onto a 96-well microplate and the remaining cell suspension was diluted 1:4, 1:16, and 1:64. Each dilution was poured onto a 96-well microplate. The cultures were maintained at 37ºC in an atmosphere containing 5% CO_2. After 48 h, 50 µL 10% S-MEM supplemented with 3X HAT (Invitrogen) was added to each well. Every 5 days, 100 µL 10% S-MEM supplemented with 1X HAT/well was exchanged. If cell proliferation was observed, cells were transferred to 24-well plates and further expanded. Two weeks after fusion, the medium was changed to 10% S-MEM supplemented with 1X HAT (Invitrogen) and subsequently changed to 10% S-MEM. Established clones were tested for reactivity in the presence of the gene.

Cytokine production by cell hybridomas

For screening, 1 x 10^5 T-cell hybridoma

cells and 1 x 10⁶ syngeneic BALB/c spleen cells were co-cultured in the presence or absence of 10 μg/mL recombinant TS in 10% S-MEM. After 3 days, the supernatants were collected and the cytokine concentration was estimated by capture ELISA using antibodies purchased from Pharmingen (San Diego, CA, USA) exactly as described by Rodrigues et al. (28).

Fluorescence-activated cell sorting analysis of the T-cell hybridomas

Phenotypic analyses of hybridomas were carried out by fluorescence-activated cell sorting (FACS) using a Facscalibur Cytometer (Becton Dickinson, Mountain View, CA, USA). Hybridomas (10⁶ cells) were incubated on ice for 45 min with hybridoma supernatants. Monoclonal antibodies anti-CD4 (GK1.5, rat IgG2), anti-CD8 (2.43, rat IgG2), anti-CD44 (KM-102, rat IgG), and anti-CD3 (145-2C11, hamster IgG) were used. After two washes, cells were incubated with fluorescein-isothiocyanate (FITC)-labeled goat anti-rat immunoglobulin G

(KPL) or FITC-labeled goat anti-hamster IgG (KPL) for an additional 45 min on ice, washed, and fixed in PBS containing 1% (w/v) paraformaldehyde. FITC-labeled anti-TCR ß chain (H57-597, Pharmingen) or anti-Vß6 TCR (RR4-7, Pharmingen, kindly provided by Dr. Milena B.P. Soares, FIOCRUZ, Brazil) was used as described above without secondary antibody incubation.

Amplification, cloning and sequencing of the α and ß variable segment genes of the TCR

Hybridoma 09 RNA was purified using TRIzol (Invitrogen) according to manufacturer instructions. RNA was precipitated with ethanol, washed, and resuspended in nuclease-free water. The NPA-PCR method was used for the amplification of TCR transcripts. Briefly, 5 μg of total RNA from hybridoma 09 was used as template for double-stranded cDNA synthesis using either the Cα3-*Not*I or Cß3-*Not*I primer (Table 1) and Superscript (Invitrogen). NPA (Table 1) were ligated onto the ends of the double-stranded cDNA, resulting in the addition of

Table 1. Primers used in the present study.

Primers	Nucleotide sequences
TCR α chain primers	
mCα3-*Not*Iª	5'- <u>TGCGGCCGCG</u>GTGAAGCTTGTCTGGTTG-3'
mCα2	5'- CCAGCACAGTTTTGTGAGTG-3'
mCα1	5'- AACAGGCAGAGGGTGCTGTCCTG-3'
mCαS (probe)	5'- CCGAGGGATCTTTTAACTGGTACACAGCAGGTTCTGGG-3'
Foward-*Sal*I α chainª	5'- <u>GTCGAC</u>ATGCACAGCCTCCTAGGG-3'
Reverse-*Bam*HI α chainª	5'- <u>GGATCC</u>TCAACTGGACCACAGCCT-3'
TCR ß chain primers	
mCß3-*Not*Iª	5'- TGCGGCCGCTGTCCTCCTCTGAAAGCCCATGGA-3'
mCß2	5'- TGCTCAGGCAGTAGCTATAATTGCTCTCC-3'
mCß1	5'- GCACACGAGGGTAGCCTTTTGT-3'
mCßS (probe)	5'- ACAAGGAGACCTTGGGTGGAGTCACATTTCTCAGATCCTC-3'
Forward-*Sal*I ß chainª	5'- <u>GTCGAC</u>ATGAACAAGTGGGTTTTCTG-3'
Reverse-*Bam*HI ß chainª	5'- <u>GGATCC</u>TCATGAATTCTTTCTTTTGAC-3'
Adaptor primer	5'- AATTCGAACCCCTTCG-3'
Ligated adaptor	5'- AATTCGAACCCCTTCG-3'
	3'- GCTTGGGGAAGCp-5'

ªUnderlined sequences represent restriction enzyme sites present in the oligonucleotides.

the adaptors at both the 5' and 3' ends. After the ligation was completed, the adaptor was removed from the 3' end by *Not*I restriction nuclease digestion, but was retained at the 5' end. A single strand of the adaptor, called adaptor primer (Table 1), was used as a sense 5' end amplification primer for both rounds of amplification performed. Two Cα primers, designated mCα2 and mCα1, and two Cß primers, designated mCß2 and mCß1 (Table 1), were used as 3' anti-sense amplification primers for the first and second amplification reactions, respectively. mCα1 and mCß1 were located 5' to the primers mCα2 and mCß2 used for the first amplification. The products resulting from the second amplification were sized ~500 bp to the TCR α chain, and ~600 bp to the TCR ß chain. Each DNA band was purified and ligated into the pMOSblue cloning vector. The transformation resulted in 24 colonies of the TCR α chain and 26 of the TCR ß chain. Twenty colonies of each chain were screened by colony hybridization with either the ^{32}P-labeled mCαS or mCßS probe (Table 1). Plasmids containing inserts representing each TCR chain were sequenced. Automatic sequencing of double-stranded DNA was performed using the BigDye Terminator Cycle Sequencing kit (Perkin-Elmer, Foster City, CA, USA) and an ABI PRISM 377 sequencer (Perkin-Elmer). U19 and T7 oligonucleotides (Invitrogen) present in the flanking regions of the pMOSblue vector were used. The comparison of the nucleic acid and the deduced amino-acid sequences obtained with the GenBank data base and NCBI/BLAST software allowed the identification of both TCR chains.

Amplification, cloning and sequencing of the genes encoding complete cDNA of the TCR α and ß chains

The cDNA reaction was performed with 5 µg of total RNA from hybridoma 09 using the ThermoScript RT-PCR system (Invitrogen). The oligo (dT) primer supplied in the kit was used for the first strand cDNA reaction. Primers specific for the variable regions and constant regions of each TCR chain (Table 1) were used to amplify the genes encoding the complete cDNA of α and ß chains. The products of amplification were sized ~800 or ~900 bp in the case of α or ß chain cDNA fragments, respectively. These PCR products were cloned into pMOSblue and sequenced.

Results

Characterization of T-cell hybridomas specific for *Trypanosoma cruzi trans*-sialidase

Twenty clones of T-cell hybridomas were established after fusion of LN cells and BWα-ß-thymoma cells. These hybridomas were stimulated *in vitro* with recombinant TS in the presence of syngeneic spleen cells. The culture supernatants were tested for the presence of cytokines. Eight hybridomas produced more than 1.2 ng/mL IFN-γ. In contrast, only one hybridoma produced 0.065 or 0.660 ng IL-4 or IL-10, respectively (Table 2). By FACS analysis, all hybridomas expressed CD4 and CD3 but none expressed the CD8 surface marker. As shown in Figure 1, T-cell hybridomas H09, H10, H21, and H26 expressed CD3 and CD4 but not CD8. All four hybridomas expressed the TCR ß chain and CD44. Control cells (BWα-ß-) did not express CD3, CD4, CD8, or TCR ß (Figure 1).

Identification of TCR transcripts by NPA-PCR

The NPA-PCR method was used for the amplification of TCR transcripts from hybridoma 09. The alignment of three clones identified the α chain as the TCRAV18S2 variable segment and TCRAJ17 junctional segment. The alignment of two clones of the ß chain identified TCRBV6S19 as the variable region and TCRBJ1S1 as the junctional region. The sequences obtained by NPA-

Table 2. Cytokine production by T-cell hybridomas after stimulation with recombinant *trans*-sialidase.

Clones	IFN-γ (ng/mL)		IL-4 (ng/mL)		IL-10 (ng/mL)	
	APC	TS	APC	TS	APC	TS
01	≤0.015	0.102 ± 0.117	≤0.015	≤0.015	≤0.015	≤0.015
05	0.072 ± 0.039	3.212 ± 0.275	≤0.015	≤0.015	≤0.015	≤0.015
08	≤0.015	1.219 ± 0.006	≤0.015	≤0.015	≤0.015	≤0.015
09	≤0.015	2.733 ± 0.043	≤0.015	≤0.015	≤0.015	≤0.015
10	0.023 ± 0.005	3.610 ± 0.099	≤0.015	≤0.015	≤0.015	≤0.015
14	≤0.015	0.596 ± 0.119	≤0.015	≤0.015	≤0.015	≤0.015
15	0.088 ± 0.024	0.829 ± 0.025	≤0.015	0.065 ± 0	≤0.015	0.660 ± 0.077
16	≤0.015	0.147 ± 0.036	≤0.015	≤0.015	≤0.015	≤0.015
18	≤0.015	0.429 ± 0.021	≤0.015	≤0.015	≤0.015	≤0.015
19	0.020 ± 0.004	0.930 ± 0.031	≤0.015	≤0.015	≤0.015	≤0.015
21	≤0.015	9.79 ± 0.127	≤0.015	≤0.015	≤0.015	≤0.015
22	0.110 ± 0.045	1.937 ± 0.019	≤0.015	≤0.015	≤0.015	≤0.015
25	2.603 ± 0.163	3.767 ± 0.273	≤0.015	≤0.015	≤0.015	≤0.015
26	≤0.015	2.119 ± 0.023	≤0.015	≤0.015	≤0.015	≤0.015
27	≤0.015	0.380 ± 0.012	≤0.015	≤0.015	≤0.015	≤0.015
28	≤0.015	0.662 ± 0.013	≤0.015	≤0.015	≤0.015	≤0.015
29	≤0.015	0.119 ± 0.013	≤0.015	0.072 ± 0.030	≤0.015	≤0.015
32	0.041 ± 0.005	0.163 ± 0.036	≤0.015	≤0.015	≤0.015	≤0.015
34	0.028 ± 0.033	0.035 ± 0.011	≤0.015	≤0.015	≤0.015	≤0.015
37	0.048 ± 0.003	0.231 ± 0.047	≤0.015	≤0.015	≤0.015	≤0.015

Data are reported as means ± SD representative of 2-4 experiments. The hybridoma clones were stimulated in the presence of antigen presenting cells (APC) and *trans*-sialidase (TS) recombinant protein (10 µg/mL) and the supernatants were tested as previously described (28).

PCR allowed us to design specific primers for the amplification of the α and ß variable regions of hybridoma 09.

cDNA from hybridoma 09 was utilized as template for RT-PCR using specific primers for the α chain gene or ß chain gene. The nucleic acid and deduced amino acid sequences revealed that the clone representing the TCR α chain transcript contained the TCRAV18S2 variable region and the TCRAJ17 junctional region (Figure 2A). The clone representing the TCR ß chain transcript contained the TCRBV6S19 variable region and TCRBJ1S1 junctional region (Figure 2B). These results confirmed the data obtained by the NPA-PCR method described above.

To confirm the presence of the TCRBV06 gene product on the surface of hybridoma 09, we stained these cells with an antibody specific for murine Vß6 (RR4-7). As shown in Figure 3, the fluorescent geometric mean of hybridoma 09 was ten times higher than in non-labeled cells or control cells (BWα-ß-). The detection of the receptor on the surface of hybridoma 09 confirmed that the ß chain gene of the TCR is in fact being functionally expressed as a protein on the surface of the hybridoma cells.

Discussion

In the present study, we established and characterized T-cell hybridomas specific for TS, an immunodominant *T. cruzi* antigen. We selected one CD3+ and CD4+ IFN-γ-secreting T-cell hybridoma to test whether we could use the NPA-PCR method to easily identify its TCR transcripts. We demonstrated that the NPA-PCR method can be useful to rapidly and efficiently determine the variable regions of both TCR transcripts of this T-cell hybridoma. These observations are important because a number of advantages
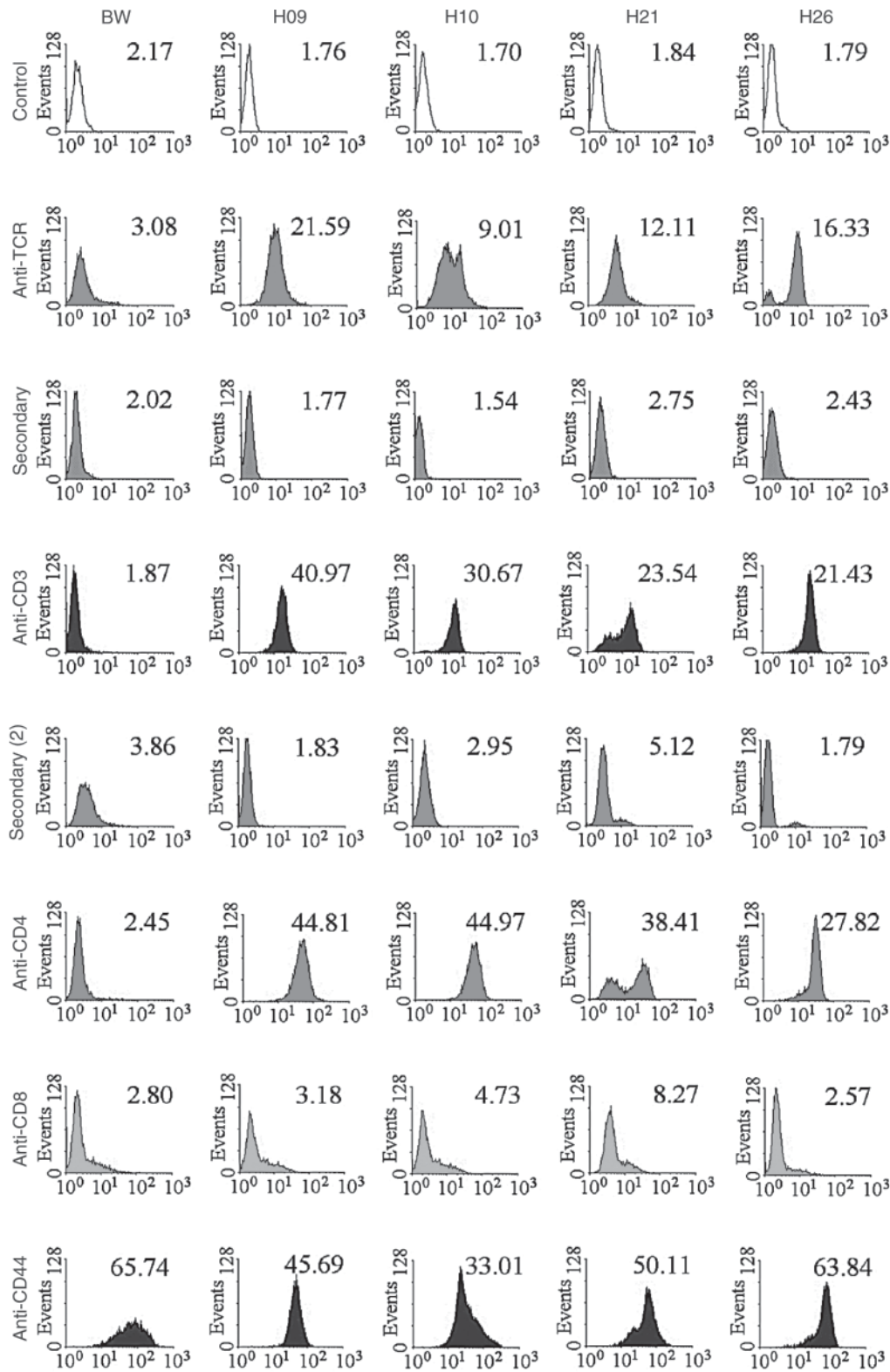
Figure 1. Expression of surface molecules in hybridomas (H) 09, 10, 21 and 26. T-cell hybridomas indicated at the top of the figure were stained with mAbs specific for the surface markers indicated, followed by FITC-conjugated goat anti-hamster (secondary) or FITC-conjugated goat anti-rat (secondary 2) antibody, and analyzed by FACS. The relative geometric mean fluorescence obtained with each mAb is indicated in the right upper corner of each histogram. TCR = T-cell receptor.

Figure 2. Deduced amino acid sequence of TCR α and ß chains from hybridoma 09. Representation of sequences obtained from TCR-H09 of α chain containing the TCRAV18S2 variable segment, TCRAJ17 junctional segment and constant region 1 (A) and ß chain containing the TCRBV6S19 variable region, TCRD diversity region, TCRBJ1S1 junctional region, and constant region (B).

A

Sal I ======================================= TRAV18S2 ======================================= 80
GTCGACATGCACAGCCTCCTAGGGTTGTTGATGGTGTCACTGTGGCTGCAACTGACAAGGGTGAATAGTCAACTAGCAGA 80
for alfa
V D M H S L L G L L M V S L W L Q L T R V N S Q L A E
AGAGAATCTGTGGGCCCTGAGCGTCCACGAGGGTGAAAGTGTCACGGTGAATTGTAGTTACAAGACATCCATAACTGCCC 160
=== TRAV18S2 ===
E N L W A L S V H E G E S V T V N C S Y K T S I T A
TACAGTGGTACAGACAGAAGTCAGGCGAAGGCCCTGCCCAGCTAATCTTAATACGTTCAAATGAGAGAGAGAAGCGCAAT 240
=== TRAV18S2 ===
L Q W Y R Q K S G E G P A Q L I L I R S N E R E K R N
GGAAGACTCAGAGCCACCCTTGACACCTCCAGCCAGAGCAGCTCCTTGTCCATCACTGCTACTCGGTGTGAAGACACCGC 320
=== TRAV18S2 ===
G R L R A T L D T S S Q S S S L S I T A T R C E D T A
TGTGTACTTCTGTGCTACTGATCAATCTTCTGGCAGCTGGCAACTCATCTTTGGATCTGGAACCCAACTGACAGTTATGC 400
=========== TRAV18S2 =========== TCRAJ17 ===========
V Y F C A T D Q S S G S W Q L I F G S G T Q L T V M
CTGACATCCAGAACCCAGAACCTGCTGTGTACCAGTTAAAAGATCCTCGGTCTCAGGACAGCACCCTCTGCCTGTTCACC 480
T ===================================== Constant Region =====================================
P D I Q N P E P A V Y Q L K D P R S Q D S T L C L F T
GACTTTGACTCCCAAATCAATGTGCCGAAAACCATGGAATCTGGAACGTTCATCACTGACAAAACTGTGCTGGACATGAA 560
=================================== Constant Region ===================================
D F D S Q I N V P K T M E S G T F I T D K T V L D M K
AGCTATGGATTCCAAGAGCAATGGGGCCATTGCCTGGAGCAACCAGACAAGCTTCACCTGCCAAGATATCTTCAAAGAA 640
=================================== Constant Region ===================================
A M D S K S N G A I A W S N Q T S F T C Q D I F K E
CCAACGCCACCTACCCCAGTTCAGACGTTCCCTGTGATGCCACGTTGACCGAGAAAAGCTTTGAAACAGATATGAACCTA 720
=================================== Constant Region ===================================
T N A T Y P S S D V P C D A T L T E K S F E T D M N L
AACTTTCAAAACCTGTCAGTTATGGGACTCCGAATCCTCCTGCTGAAAGTAGCGGGATTTAACCTGCTCATGACGCTGAG 800
=================================== Constant Region ===================================
N F Q N L S V M G L R I L L L K V A G F N L L M T L R
GCTGTGGTCCAGTTGAGGATCC 822
Constant Region | BamH I
rev alfa
L W S S . G S

B

Sal I ======================================= TCRBV6S19 ======================================= 80
GTCGACATGAACAAGTGGGTTTTCTGCTGGGTAACCCTTTGTCTCCTTACTGTAGAGACCACACATGGTGATGGTGGCAT 80
for beta
V D M N K W V F C W V T L C L L T V E T T H G D G G I
CATTACTCAGACACCCAAATTCCTGATTGGTCAGGAAGGGCAAAAACTGACCTTGAAATGTCAACAGAATTTCAATCATG 160
=== TCRBV6S19 ===
I T Q T P K F L I G Q E G Q K L T L K C Q Q N F N H
ATACAATGTACTGGTACCGACAGGATTCAGGGAAAGGATGAGACTGATCTACTATTCAATAACTGAAAACGATCTTCAA 240
=== TCRBV6S19 ===
D T M Y W Y R Q D S G K G L R L I Y Y S I T E N D L Q
AAAGGCGATCTATCTGAAGGCTATGATGCGTCTCGAGAGAAGAAGTCATCTTTTTCTCTCACTGTGACATCTGCCCAGAA 320
=== TCRBV6S19 ===
K G D L S E G Y D A S R E K K S S F S L T V T S A Q K
GAACGAGATGGCCGTTTTTCTCTGTGCCAGCAGTATAACGGACACAAACACAGAAGTCTTCTTTGGTAAAGGAACCAGAC 400
=========== TCRBV6S19 =========== D === TCRBJ1S1 ===========
N E M A V F L C A S S I T D T N T E V F F G K G T R
TCACAGTTGTAGAGGATCTGAGAAATGTGACTCCACCCAAGGTCTCCTTGTTGAGCCATCAAAAGCAGAGATTGCAAAC 480
TCRBJ1S1 === Constant Region1 ===
L T V V E D L R N V T P P K V S L F E P S K A E I A N
AAACAAAAGGCTACCCTCGTGTGCTTGGCCAGGGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGCAA 560
=================================== Constant Region1 ===================================
K Q K A T L V C L A R G F F P D H V E L S W W V N G K
GGAGGTCCACGTGGGGTCAGCACGGACCCTCAGGCCTACAAGGAGAGCAATTATAGCTACTGCCTGAGCAGCCGCCTGA 640
=================================== Constant Region1 ===================================
E V H S G V S T D P Q A Y K E S N Y S Y C L S S R L
GGGTCTCTGCTACCTTCTGGCACAATCCTCGCAACCACTTCCGCTGCCAAGTGCAGTTCCATGGGCTTTCAGAGGAGGAC 720
=================================== Constant Region1 ===================================
R V S A T F W H N P R N H F R C Q V Q F H G L S E E D
AAGTGGCCAGAGGGCTCACCCAAACCTGTCACACAGAACATCAGTGCAGAGGCCTGGGGCCGAGCAGACTGTGGGATTAC 800
=================================== Constant Region1 ===================================
K W P E G S P K P V T Q N I S A E A W G R A D C G I T
CTCAGCATCCTATCAACAAGGGGTCTTGTCTGCCATCCATCCTCTATGAGATCCTGCTAGGGAAAGCCACCCTGTATGCTG 880
=================================== Constant Region1 ===================================
S A S Y Q Q G V L S A T I L Y E I L L G K A T L Y A
TGCTTGTCAGTACACTGGTGGTGATGGCTATGGTCAAAAGAAAGAATTCATGAGGATCC 939
=================================== Constant Region1 === Bam HI
rev beta
V L V S T L V V M A M V K R K N S . G S

can be credited to this methodology. First, the NPA-PCR method uses the same reagents (primers) for the identification of TCR transcripts of any T-cell clone or hybridoma. Second, these primers are much more reliable and less expensive than the specific monoclonal antibodies used for the identification of TCR. Third, because a number of antibodies specific for the TCR Vα families are not available, the screening using specific antibodies may end up being useless. Finally, this method does not require the laborious generation and screening of cDNA libraries.

On the basis of these demonstrated advantages, we believe that this methodology can significantly contribute to the identification of TCR from T-cell hybridomas or clones, that can be used to generate TCR-transgenic mice. More specifically, in our case, the TCR genes isolated from hybridoma 09 may allow us to establish a TCR transgenic mouse specific for an immunodominant *T. cruzi* antigen.
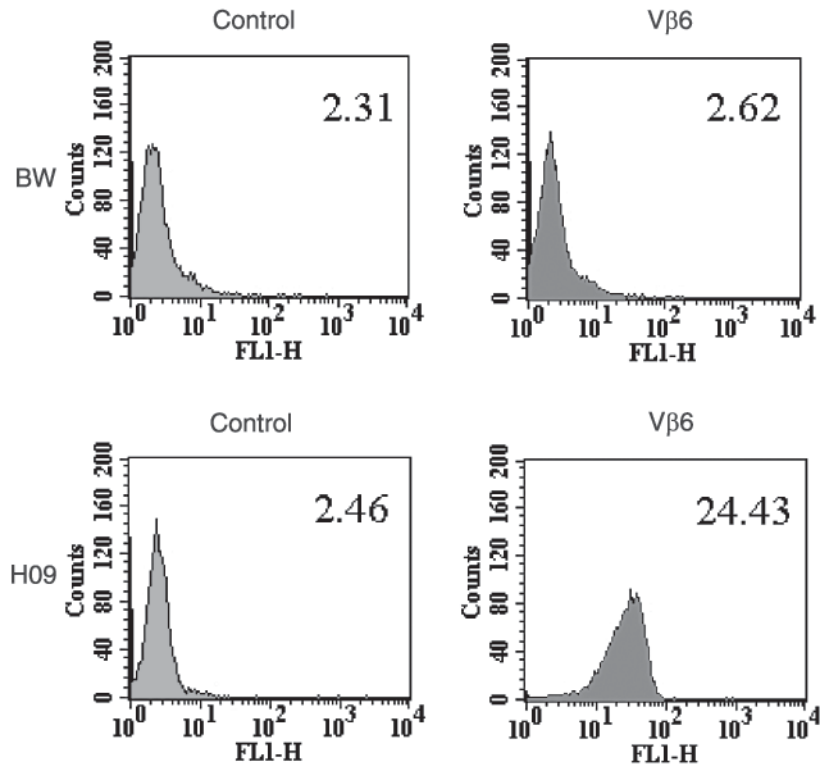

Figure 3. Expression of Vß6 on the surface of hybridoma (H) 09. H09 and BWα-ß-cells were stained with an FITC-labeled mAb specific for murine Vß6. The relative geometric mean fluorescences are indicated in the right upper corner of each histogram.

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