Novel SLA-DR Alleles of Three Chinese Pig Strains and the Related Function in Human T Cell Response

Fuxiang Chen[1, 3], Jin Xie[1], Yun Zhou[1, 2], Ningli Li[1, 2] and Kuang-Yen Chou[1, 2, 4]

To elucidate the structures of SLA-DR (swine leukocyte antigen DR) genes of three Chinese pig strains (Gz, Bm and Yn), the SLA-DRA and SLA-DRB cDNA were amplified by RT-PCR and subjected to determine the sequences. The whole structures of SLA-DRA alleles are identical among three strains, consisting of 759 nucleotides including an open reading frame (ORF), and are shared with those reported from NIH minipigs SLA-DRA^c and SLA-DRA^d. The same length of the ORF-containing SLA-DRB genes of three Chinese pig strains was also identified. They are composed of 801 nucleotides encoding a xenogeneic antigen molecule of 266 amino acid residues. The nucleotide sequences of the SLA-DRB genes, however, are different when compared either among the three strains or with the published data of SLA-DRB sequences, which allowed our novel SLA-DRB alleles receiving their accession numbers AY102479, AY102480 and AY102481 from the GenBank. This study further reveals that the phylogenic homologies of MHC DR or DR-like genes in structures of nucleotides and deduced amino acids between Chinese pigs (SLA) and human (HLA-DRB1*0901) are better than those between pigs and mice (H-2^b Eβ). High similarities were also found for DRα-DRβ heterodimers between Chinese pigs and human in terms of amino acids sequences critical for binding with human CD4 coreceptor molecule, which are better than those between SLA-DR and H-2 I-E molecules. A functional test indicated that, by cotransfection with Bm-DRA and Bm-DRB genes, the Bm-DR molecule-expressed L929 cells could stimulate human T cells quite well in a xenogeneic reaction in presence of human APCs. *Cellular & Molecular Immunology*. 2004;1(3):212-218.

Key Words: MHC-DR, SLA, Chinese pig strain, CD4 coreceptor, xenotransplantation

Introduction

Discordant xenografting using pigs as organ donors has been regarded one of the most hopeful approaches to solve the shortage of organ grafts in clinic transplantation (1, 2). The successful overcoming the hyper acute rejection (HAR) by constructing the transgenic swine with human DAF gene (3, 4) and developing the α-1, 3-galactosyltransferase gene knockout pigs with nuclear transfer cloning approach (5) have pushed the investigation further towards the following rejection responses, the delayed xenografts rejection (DXR) and the acute cellular rejection (ACR). It is thus of importance to elucidate the structures of swine leukocyte antigen (SLA) genes, the swine MHC, from various pig strains since either cytotoxic activity of NK cells in DXR (6) or cellular rejection in ACR all depends on expression of SLA on porcine endothelium (2, 7, 8).

SLA molecules are encoded by a group of tightly linked genes located on 7p1.1 and 7q1.1 of swine chromosome (9). There are SLA gene loci correspondent to HLA-DRA and HLA-DRB. In China, pigs are traditionally bred in a highly isolated condition. It is thus valuable to select those from Chinese pig strains with satisfied immunogenetic properties to use as candidates for xenotransplantation.

Based on the structures of the NIH minipig class II genes, SLA-DR cDNA of three Chinese pig strains were cloned and amplified by RT-PCR in this study. Data we obtained indicated that our pig strains were all endowed with new sequences for their DRB alleles. Some related experiments were also performed to functionally confirm that the Chinese swine DR molecules were capable of evoking a human T cell response in xenogeneic reactions.

[1] Shanghai Institute of Immunology, Shanghai Second Medical University, Shanghai 200025, China.

[2] E-Institutes of Shanghai Universities, Immunology Division, Shanghai 200025, China.

[3] The Oriental Hospital, Tongji University, Shanghai 200120, China.

[4] Corresponding to: Dr. Kuang-Yen Chou, Shanghai Institute of Immunology, Shanghai Second Medical University, 280 South Chongqing Road, Shanghai 200025, China. Fax: +86-21-63846383, E-mail: my@shsmu.edu.cn.

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Abbreviations: HAR, hyper acute rejection; DXR, xeno-grafts rejection; ACR, acute cellular rejection; SLA, swine leukocyte antigen; ORF, open reading frame; MLR, mixed leukocyte reaction.

Materials and Methods

Pig strains

Three Chinese miniature pig strains used for our studies were offered by the Shanghai Experimental Animal Center, Chinese Academic of Sciences. They are Guizhou Xiang pig (Gz), Bama pig (Bm, formally called Guangxi pig, Gx) and Yunan Banna pig (Yn). Gz and Bm are strains properly inbred with an intra-strain similarity coefficient 0.928 and 0.933, respectively (10). Yn pig is under its way to become an inbred Banna minipig strain with a high inbreeding coefficient (11).

Isolation and amplification of swine SLA-DR cDNA

Following the manufacturer's protocol (Gibco BRL), total RNA extracted from PBMCs of the Chinese pigs using TRIzol reagent was converted to cDNA by the procedure with oligo-(dT) primers (Promega, Madison, WI). The resultant cDNA was used as PCR template. Based on the genomic sequences of GenBank database for DRA and DRB of NIH minipig (12, 13), two pairs of primers were designed and synthesized with software Primer Express 3.0. DRA: up- 5'ATC GAA GCT TCT CAT CGA GGC ATC TAA GGA G; down- 5'CGA TCT CGA GCT GCA AGC ACC TCA CAG AG. DRB: up- 5'ATC GAA GCT TCT CTC CTG TTC TTC AGC ATG; down- 5'CGA TCT CGA GCA CCA CCT GGA CCT CAG CTC. The sites for restriction enzymes Hind III (AAGCTT) and XhoI (CTCGAGT) are inserted and indicated by underlines.

Amplification reactions were performed in 50 μl mixture containing 20 U/ml Taq polymerase, 200 μM deoxynucleotide triphosphate, 0.2 μM of the two primers and 1.5 mM Mg^{2+}. The PCR cycles were 60 sec denaturing at 94°C, 90 sec annealing at 55°C and 60 sec extension at 72°C. The reaction was performed for 30 cycles ending with a step of 7 min at 72°C.

Cloning and sequencing of SLA-DRA and SLA-DRB cDNA

PCR products were purified with QIAquick Gel Extraction (Qiagen, Chatswoth, CA) and inserted into pGEM-T Easy vector (Promega, Madison, WI). The DR cDNA clones were analyzed by restriction mapping as follows: 1 μg of DNA (DR cDNA clones in pGEM-T Easy vector) was digested with Hind III and XhoI at 37°C for 2 h. Products were separated on 0.8% agarose gels and stained with ethidium bromide. The nucleotide sequences for positive recombinant plasmids were determined by the dideoxy chain-termination method. Two directions sequencing reactions were performed on 9600 thermal reactor (Perkin-Elmer) using Big Dye Terminator Cycle Sequencing Chemistries with universal SP6 and T7 primers. Reaction products were electrophoresed on a 377 DNA sequencer (Perkin-Elmer) and sequence data were processed by means of Data Collection on a Macintosh personal computer. Auto-assembler (Perkin-Elmer) was applied to assemble the sequences and DNA. Strider (version 1.2) was employed to analyze the open reading frame (ORF) of pig DR cDNA. These sequences were searched against GenBank databases for homology comparison using BLAST in the Genetics Computer Group Program package.

Expression of SLA-DR molecule on L929 cells

The SLA-DRA/DRB genes (Bm-DRA and Bm-DRB) were inserted into vector pcDNA 3.0 at Hind III/Xho site to construct the recombinant expressing plasmids Bm-DRA-pcDNA and Bm-DRB-pcDNA. Mouse L929 fibroblast cells were cotransfected with the combination of Bm-DRA-pcDNA/Bm-DRB-pcDNA. Control cells were transfected with vector pcDNA3.0 alone. Transfection was carried out by using lipofectamine reagent, followed by cell selection in G418-containing medium. Some DRA/DRB-transfected clones were isolated by limited dilution. The expression of DR molecule on L929 was determined by flow cytometry (FACScan, Becton Dickinson) with fluorescence-labeled anti-SLA-DR mAb (PharMingen).

One-way MLC for functional determination of lymphoproliferation to SLA-DR-expressed cells

8000 Rad-irradiated L929 cells, transfected with Bm-DRA/DRB genes or empty vector, were seeded onto round bottomed 96-well culture plates (Costar) at concentration 2×10^4/well and cultured in DMEM medium for 24 hours. Human PBMCs were freshly isolated, washed and suspended at concentration 1.6×10^6/ml in RPMI 1640 culture medium, supplemented with 2 mM glutamine, 10 mM acetonate natrium, 10 mM HEPES, 100 IU/ml Penicillin, 100 μg/ml Streptomycin and 5% pooled human AB sera. After removing the original culture medium from the wells in which L929 cells had been seeded, the PBMC suspension was added to each well at an amount of 0.1 ml per well. In antibody blocking experiments, the L929 cells were pulse-treated for 30 min with anti-SLA-DR mAb or unrelated anti-human CD3 mAb before seeding on the plates. The mixed cells in suspension were cultured at 37°C with 5% CO_2 for 5 days. Sixteen hours before termination of the culturing, 1 μCi of ^{3}H-TdR (specific activity 32 Ci/mM, Shanghai Nuclear Institute) was added into each well. Isotope uptake was determined by liquid scintillation counter (Rackbeta 1290, Pharmacia) and expressed as count per minute (cpm). Stimulation index (SI) was calculated: SI = cpm of exp combination / cpm of negative control. SI ≥ 3.0 is set as a cut-off point to indicate an effective xenogeneic response.

Results

Analysis of the SLA-DR cDNA from Chinese pig strains

Clear RT-PCR amplified bands of SLA-DRA and SLA-DRB cDNA are visualized in electrophoresis on agarose gel (Figure 1A). The sizes of amplified products of two DR genes are nearly identical, round 800 bp as expected. The Figure 1 only shows the results from Gz pig as an example since identical results were obtained for Bm and Yn pigs.

After purification, the PCR products were inserted into pGEM-T Easy vector and digested with Hind III and Xho I, followed by separating on agarose gel. Figure 1B presents the related results of electrophoresis for SLA-DR genes of Gz pig, including the DRA- and DRB-containing recombinant plasmids (lane 1 and 2) and their products digested with the restriction endonucleases (lane 3 and 4). For the later, there are two bands appeared in each lane for the

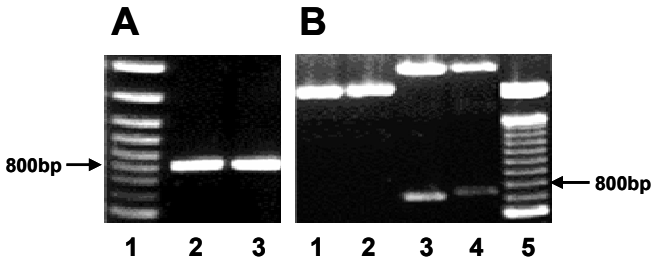

Figure 1. RT-PCR-amplified SLA-DR genes of Chinese Gz pig. (A) Electrophoresis patterns of the RT-PCR-amplified Gz-DRA and Gz-DRB cDNA. The sizes of the amplified products are round 800 bp as indicated in electrophoreses on agarose gel. (lane 1, 100 bp marker; lane 2, Gz-DRB; lane 3, Gz-DRA.) (B) Electrophoresis patterns of the restriction enzyme-digested Gz-DR cDNA clones. The PCR products were inserted into pGEM-T vector and digested with Hind III and Xhol, followed by separating on agarose gel. As shown in lane 3 and 4, there are two bands for vector and the amplified products respectively, indicating a successful ligation of Gz-DR cDNA with the sequencing vector. (lane 1, Gz-DRA-pGEM-T; lane 2, Gz-DRB-pGEM-T; lane 3, Gz-DRA-pGEM-T/ Hind III + Xho I; lane 4, Gz-DRB-pGEM-T/Hind III + Xho I; lane 5, 100 bp marker.)

vector and the amplified products (round 800 bp), respectively, indicating a successful ligation of SLA-DR cDNA with the sequencing vector. There are similar results for the other two Chinese pig strains (data and figure not shown).

Sequence analysis for SLA-DR genes of Chinese pig strains

Sequencing reactions of two directions were performed with universal SP6 and T7 primers. Processed with computer, the sequencing data were assembled and subjected to work out the ORF-containing SLA-DRA cDNA sequences for three Chinese pig strains. As indicated in Figure 2, the 759 nucleotides-composed SLA-DRA allele, which is identical for all three Chinese pig strains, encodes for a polypeptide of 252 amino acid residues. The polypeptide includes a signal sequence (aa 1-23), α1 domain (aa 24-107), α2 domain (aa 108-202) and a membrane/ plasma fragment (aa 203-252). Homologies among Gz-DRA, Bm-DRA and Yn-DRA are 100% both at nucleotide level and amino acid level. And they share same sequence with the "standard" NIH minipigs (13). The invariable pattern of the SLA-DRA gene structure might reflect its nature with little polymorphism as identified in human HLA-DRA1 locus. For this reason only the sequence of Gz-DRA gene is given.

In contrast, three SLA-DRB alleles, all with 801 nucleotides that encode a polypeptide of 266 amino acid residues, were polymorphic for Gz-DRB, Bm-DRB and Yn-DRB loci (Figure 3). The relevant polypeptides are structurally comparable, each containing a signal sequence (aa 1-29), α1 domain (aa 30-123), α2 domain (aa 124-217) and a membrane/plasma fragment (aa 218-266). The sequence homologies among Bm-DRB, Gz-DRB and

Table 1. *A comparison of the human CD4-binding amino acid sequences of the second domains of MHC-DRα chains among human, pig and mouse.*

	Amino acid residue[2]												
	124	125	126	127	128	129	130	131	132	133	134	135	136
HLA-DRA1 *0101	N	G	K	P	V	T	T	G	V	S	E	T	V
SLA-DRA[1]	-	-	S	-	-	-	R	-	-	-	-	-	-
H-2^{b}Eα	-	S	-	S	-	-	D	-	-	V	-	-	S

[1] Since the whole sequence are identical for Gz-DRα, Bm-DRα and Yn-DRα chains, only the related sequence of Gz-DRα chain is presented as an example.
[2] The sequence 124-136 corresponds to 147-159 of the signal peptide-containing sequence of Gz-DRA presented in Figure 2B.

Table 2. *A comparison of the human CD4-binding 136–148 amino acid sequences of the non-polymorphic second domains of MHC-DRβ chains among human, pig and mouse.*

	Amino acid residue[2]												
	136	137	138	139	140	141	142	143	144	145	146	147	148
HLA-DRB1 *0901	Q	E	E	K	A	G	V	V	S	T	G	L	I
Gz-DRB[1]	-	-	-	A	-	-	-	-	-	-	-	-	-
H-2^{b}Eβ	R	-	-	-	T	-	I	T	-	-	-	-	V

[1] Since the non-polymorphic second domains of Gz-DRβ, Bm-DRβ and Yn-DRβ chains are identical in their sequences for binding with human CD4 molecule, only the relevant sequence of Bm-DRB is presented as an example.
[2] The sequence 136-148 corresponds to 165-177 of the signal peptide-containing sequence of Gz-DRA as presented in Figure 2B.

(A) Gz-DRA

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  1 ATGACCATAC TTGGGGTCCC AGTCCTGGGA TTTGTCATCA CCATCTTGAA
 51 CCTTCAGAAA TCATGGGCTA TCGTAGAGAA TCACGTGATC ATCCAGGCTG
101 AGTTCTATCT GAGCCCTGAC AAATCTGGCG AGTTTATGTT TGACTTTGAC
151 GGTGATGAGA TTTTCCACGT GGATATGGAA AAGAGGGAGA CGGTCTGGCG
201 ACTTGAAGAA TTTGGACATT TTGCCAGCTT TGAGGCTCAG GGTGCACTGG
251 CCAACATAGC TGTGGACAAA GCCAACCTGG AAATCCTGAT CAAGCGCTCC
301 AACAACACCC CGAACACCAA TGTACCTCCA GAAGTGACTG TGCTCTCAGA
351 CAAGCCTGTT GAACTGGGAG AGCCCAACAT CCTCATCTGT TTCATCGACA
401 AGTTCTCCCC GCCAGTGGTC AATGTCACCT GGCTTCGAAA TGGCTCCCCT
451 GTCACCAGAG GAGTGTCAGA GACAGTCTTC CTGCCCCGGG AGGACCACCT
501 TTTCCGCAAG TTCCACTATC TCCCCTTCAT GCCCTCAACC GAGGATGTCT
551 ATGACTGCCA GGTGGAGCAC TGGGGTTTGG ACAAGCCTCT TCTCAAGCAC
601 TGGGAGTTTG AAGCTCAAAC CCCCCTCCCA GAGACTACAG AGAACACCGT
651 GTGTGCTCTG GGCCTGATTG TGGCTCTGGT GGGCATCATC GTCGGGACCG
701 TCCTCATCAT CAAGGGTGTG CGCAAAGGCA ACGCCACTGA ACGCCGAGGG
751 CCTCTGTGA
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(B) Gz-DRA

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  1 MTILGVPVLG FVITILNLQK SWAIVENHVI IQAEFYLSPD KSGEFMFDFD
 51 GDEIFHVDME KRETVWRLEE FGHFASFEAQ GALANIAVDK ANLEILIKRS
101 NNTPNTNVPP EVTVLSDKPV ELGEPNILIC FIDKFSPPVV NVTWLRNGSP
151 VTRGVSETVF LPREDHLFRK FHYLPFMPST EDVYDCQVEH WGLDKPLLKH
201 WEFEAQTPLP ETTENTVCAL GLIVALVGII VGTVLIIKGV RKGNATERRG
251 PL*
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Figure 2. The sequence of SLA-DRA allele that is identical to three Chinese pig strains. (A) cDNA sequence; (B) Amino acid sequence deduced from the cDNA sequence.

(A)

```
          1                                                                                                   100
Bm-DRB    ATGTTGCATC TGTGTTTCTC CAGAGGCTTT AGGATGGTGG CTCTGACCGT GATGCTGGTG GTGCTGAGCC CTCCCTTGGC TTTGGCCAGG GACACCCCAC
Gz-DRB    ATGTTGCATC TGTGTTTCTC CAGAGGCTTC TGGATGGCAG CTCTGACTGT GATGCTGGTG GTGCTGAGCC CTCCCTTGGC TTTGGCCAGG GACACCCCAC
Yn-DRB    ATGTTGCATC TGTGTTTCTC CAGAGGCTTC TGGATGGCGG CTCTGACCGT GATGCTGGTG GTGCTGAGCC CTCCCTTGGC TTTGGCCAGG GACACCCCAC
          101                                                                                                 200
Bm-DRB    CGCATTTCTT GCACCTGGTG AAACACGAGT GTCGTTTCTT CAACGGGACG GAGCGGGTGC TGTTGCTGGA CAGATACTTC TATAACGGAG AGGAGTTCGT
Gz-DRB    CGCATTTCTT GTTTCTGGGG AAATTCGAGT GTCATTTCTT CAATGGGACC GAGCAGGTGA GGTTATTGGA GAGGCAATAC TATAACGGAG AGGAGTTCGT
Yn-DRB    CGCATTTCTT GCTTCTGGTG AAATCCGAGT GTCATTTCTT CAACGGGACC GAGCGGGTGA GGTTCATGGA GAGGCACTAC TATAACGGAG AGGAGCACGT
          201                                                                                                 300
Bm-DRB    GCGCTTCGAC AGCGACGTGG GCGAGTTCCG GGCGGTGACC GAGCTGGGGC GGCCAGACGC CAAGTACTGG AACAGCCAGA AGGACATCCT GGAGGACTCA
Gz-DRB    GCGCTTCGAC AGCGACGTGG GCGAGTACCG GGCGGTGACC GAGCTGGGGC GGCCAGACGC CAAGAACTAT AACAGCCAGA AGGACCTCCT GGAGCAGAGG
Yn-DRB    GCGCTTCGAC AGCGACGTGG GCGAATACCG GGCGGTGACC GAGCTGGGGC GGCCTTCAGC CAAGTACTGG AACAGCCAGA AGGACATCCT GGAGGAGAAG
          301                                                                                                 400
Bm-DRB    CGGGCCTCAG TGGACACGTA CTGCATACAC AACTACAGGA TTTTGGATAC ATTCCTCGTG CCGCGGCGAG CTGAGCCCAC GGTGACGGTG TACCCTGCAA
Gz-DRB    CGGGCGGAGG TGGACACGTA CTGCAGACAC AACTACAGGA CCTCCGATAC ATTCCTGGTG CCGCGGCGAG CTGAGCCCAG AGTGACGGTG TATCCTGCAA
Yn-DRB    CGGGCGGAGG TGGACACGTA CTGCAGACAC AACTACAGGA CCTCGGATAC ATTCCTGGTG CCGCGGCGAG CTGAGCCCAG AGTGACGGTG TACCCTGCAA
          401                                                                                                 500
Bm-DRB    AGACCCAGCC CCTGCAGCAC CACAACCTCC TGGTCTGCTC TGTGACCGGG TTCTACCCAG GCCACGTGGA GGTCAGGTGG TTCCGGAATG GCCAGGAAGA
Gz-DRB    AGACCCAGCC CCTGCAGCAC CACAACCTCC TGGTCTGCTC TGTGACCGGG TTCTACCCAG GCCACGTGGA GGTCAGGTGG TTCCGGAATG GCCAGGAAGA
Yn-DRB    AGACCCAGCC CCTGCAGCAC CACAACCTCC TGGTCTGCTC TGTGACCGGG TTCTACCCAG GCCGCGTGGA GGTCAGGTGG TTCCGGAATG GCCAGGAAGA
          501                                                                                                 600
Bm-DRB    GGCGGCGGGG GTGGTCTCCA CAGGCCTGAT CCCTAATGGA GACTGGACCT TCCAGACCAT GGTGATGCTT GAAACGGTTC CTCAGAGTGG AGAGGTCTAC
Gz-DRB    GGCGGCGGGG GTGGTCTCCA CAGGCCTGAT CCCTAATGGA GACTGGACCT TCCAGACCAT GGTGATGCTT GAAACGGTTC CTCAGAGTGG AGAGGTCTAC
Yn-DRB    GGCGGCGGGG GTGGTCTCCA CAGGCCTGAT CCCTAATGGA GACTGGACCT TCCAGACCAT GGTGATGCTT GAAACGGTTC CTCAGAGTGG AGAGGTCTAC
          601                                                                                                 700
Bm-DRB    AGCTGCCGAG TGGAGCACCC CAGCCTGACG AGCCCCGTCA CAGTGGAATG GAGGGCACGG TCTGAATCTG CTCAGGGCAA GATGATGAGT GGGATCGGGG
Gz-DRB    AGCTGCCGAG TGGAGCACCC CAGCCTGACG AGCCCCGTCA CAGTGGAATG GAGGGCACGG TCTGAATCTG CTCAGGGCAA GATGATGAGT GGGATCGGGG
Yn-DRB    ACCTGCCGAG TGGAGCACCC CAGCCTGACG AGCCCCGTCA CAGTGGAATG GAGGGCACGG TCTGAATCTG CTCAGGGCAA GATGATGAGT GGGGTCGGGG
          701                                                                                                 800
Bm-DRB    GCTTCGTCCT GGGTCTGCTC TTTGTTGCTG TGGGGCTGTT CATCTACTTC AAGAATCAGA AAGGACGCCC TGCCCTTCAG CCAACAGGCC TCCTGAGCTG A
Gz-DRB    GCTTCGTCCT GGGTCTGCTC TTTGTTGCTG TGGGGCTGTT CATCTACTTC AAGAATCAGA AAGGACGCCC TGCCCTTCAG CCAACAGGCC TCCTGAGCTG A
Yn-DRB    GCTTCGTCCT GGGTCTGCTC TTTGTTGCTG TGGGGCTGTT CATCTACTTC AAGAATCAGA AAGGACGCCC TGCCCTTCAG CCAACAGGCC TCCTGAGCTG A
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(B)

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          1                                                                                                   100
Gz-DRB    MLHLCFSRGF WMAALTVMLV VLSPPLALAR DTPPHFLFLG KFECHFFNGT EQVRLLERQY YNGEEFVRFD SDVGEYRAVT ELGRPDAKNY NSQKDLLEQR
Yn-DRB    MLHLCFSRGF WMAALTVMLV VLSPPLALAR DTPPHFLLLV KSECHFFNGT ERVRFMERHY YNGEEHVRFD SDVGEYRAVT ELGRPSAKYW NSQKDILEEK
Bm-DRB    MLHLCFSRGF RMVALTVMLV VLSPPLALAR DTPPHFLHLV KHECRFFNGT ERVLLLDRYF YNGEEFVRFD SDVGEFRAVT ELGRPDAKYW NSQKDILEDS
          101                                                                                                 200
Gz-DRB    RAEVDTYCRH NYRTSDTFLV PRRAEPRVTV YPAKTQPLQH HNLLVCSVTG FYPGHVEVRW FRNGQEEAAG VVSTGLIPNG DWTFQTMVML ETVPQSGEVY
Yn-DRB    RAEVDTYCRH NYRTSDTFLV PRRAEPRVTV YPAKTQPLQH HNLLVCSVTG FYPGRVEVRW FRNGQEEAAG VVSTGLIPNG DWTFQTMVML ETVPQSGEVY
Bm-DRB    RASVDTYCIH NYRILDTFLV PRRAEPTVTV YPAKTQPLQH HNLLVCSVTG FYPGHVEVRW FRNGQEEAAG VVSTGLIPNG DWTFQTMVML ETVPQSGEVY
          201                                                 267
Gz-DRB    SCRVEHPSLT SPVTVEWRAR SESAQGKMMS GIGGFVLGLL FVAVGLFIYF KNQKGRPALQ PTGLLS*
Yn-DRB    TCRVEHPSLT SPVTVEWRAR SESAQGKMMS GVGGFVLGLL FVAVGLFIYF KNQKGRPALQ PTGLLS*
Bm-DRB    SCRVEHPSLT SPVTVEWRAR SESAQGKMMS GIGGFVLGLL FVAVGLFIYF KNQKGRPALQ PTGLLS*
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Figure 2. The sequences of SLA-DRB alleles of three Chinese pig strains. (A) cDNA sequences; (B) Amino acid sequences deduced from the cDNA sequence.

Yn-DRB are 94.1-96.5% at nucleotide level and 91.0-93.6% at amino acid level.

An effort of searching identical alleles from other published SLA-DRB gene sequences, including NIH minipigs, was not successful (data not shown). A further comparison of homology with GenBank databases was performed by using BLAST in the Genetics Computer Group Program package. Our three SLA-DRB alleles could thus be assigned as novel genes with GenBank accession numbers AY102479 - AY102481.

Phylogeneic comparison analysis of MHC-DR genes among Chinese pigs, human and mice

It is of interest to note that much sequence discrepancies are existed for DRA genes when comparing our SLA-DRA with HLA-DRA1, the allele has been identified in Chinese population (14), or with mouse H-2 Eα gene. The DRA homologies between our SLA and HLA are 87.1% and 83.4% at nucleotide and amino acid level respectively, which is much better than the homologies of 64.5% and 53.4% at DNA and protein levels between our SLA-DRA and the mouse DRA-like gene Eα. A phylogenic tree of DRA genes was drawn based on the homology analysis with GeneDoc software and GCG package, confirming a nearer evolution distance between SLA-DRA and HLA-DRA molecules than that between SLA-DRA and mouse Eα molecule (data and figure not presented).

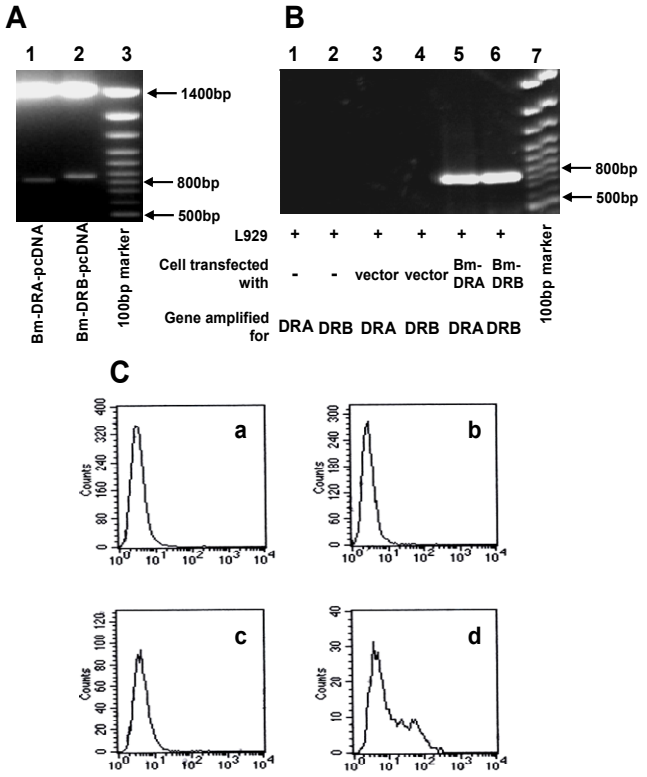

Figure 4. Transfection of L929 cells with SLA-DR genes of Chinese Bm pig strain. (A) Identification of the recombinant expressing plasmids with restriction enzymes for Bm-DRA/DRB genes. (B) Determination of SLA-DR gene expression on L929 cells by RT-PCR. Lane 5 and 6 indicate a successful transfection with Bm-DRA and Bm-DRB genes into L929 cells. (C) Determination of SLA-DR molecule expression on L929 cells by flow cytometry. (a) Untreated L929 cells; (b) Empty vector-transfected L929 cell clone; (c) The L929 clone transfected with Bm-DRA and Bm-DRB genes detected with unrelated mAb; (d) The L929 clone transfected with Bm-DRA and Bm-DRB genes detected with anti-SLA-DR mAb.

Furthermore, the homologies of DRB sequences are 93.6-97.8% and 89.9-96.6% at nucleotide and amino acid levels respectively when comparing Chinese pigs (Bm-DRB, Gz-DRB and Yn-DRB) with NIH minipigs (SLA-DRB^c and SLA-DRB^d) (14). In case the SLA-DRB alleles of Chinese pigs were set for comparison with some HLA-DRB1 alleles that are popular in Chinese Han population, e.g., DRB1*0901, DRB1*1101 and DRB1*1201 (14), the sequence homologies drop to 81.4-82.7% and 73.0-76.4% at DNA and protein levels. The fact that there are more inter-species discrepancies might account for the stronger immune responses of human T cells to swine MHC antigens than to allogeneic ones. When H-2^b Eβ, a DRB-like allelic molecule, was further included for comparison, the sequence homologies reduced to 78.4-79.2% and 70.2-72.4% for nucleotides and amino acids, respectively.

The sequences of SLA-DR chains corresponding to their HLA counterparts recognized by human coreceptor CD4 molecule

A group of membrane molecules are active in antigen recognition and signal transduction of T cells. Among them, coreceptor CD4 molecule, a member of Ig superfamily with four Ig-like domains in its out-membrane part, is especially critical. By binding with the non-polymorphic sites of MHC class II molecules, CD4 molecule functions to stabilize the interactions of MHC-peptide-TCR and to join the initiation of T cell signaling *via* the CD4-associated Src-PTK molecules (15, 16). The critical amino acid residues on HLA-DRα and -DRβ chain for binding with human CD4 molecule are located on position 124 to 136 and 134 to 148, respectively (17). It is thus of importance to determine the relevant structures in SLA-DRα and -DRβ chain of Chinese pig strains in case a pig to human xenotransplantation is set. As indicated in Table 1, for DRA molecules there are only two amino acid residues exchanged as Lys126Ser and Thr130Arg when HLA-DRα chain (DRA1*0101) is compared with our SLA-DRα chain. In contrast, there are five positions of amino acid residues substituted when the HLA-DRα chain is compared with H-2 Eα: Gly125Ser, Pro127Ser, Thr130Asp, Lys133Tyr and Val136Ser.

Table 2 gives the results of amino acid sequence comparison for DRβ chains. In HLA-DRβ (DRB1*0901) *vs.* SLA-DRβ (Gx-DRβ), there is only one position of amino acid residue exchanged: Lys139Ala. For HLA-DRβ (DRB1*0901) *vs.* H-2^b Eβ, however, four positions show amino acid substitutions: Glu136Lys, Ala140Thr, Val142 Ile and Ile148Val.

Table 3. *Human lymphoproliferation to SLA-DR gene-transfected cells in one-way MLC.*

Responder	Stimulator[2]	Gene Transfected	Blocking mAb	Exp 1		Exp 2	
				cpm	SI	cpm	SI
hPBMC[1]	L929-PC18[3]	/	/	592 ± 148	1.0	546 ± 57	1.0
		/	unrelated	691 ± 71	1.2	593 ± 65	1.1
hPBMC	L929-DR24[4]	SLA-DRA/DRB	/	6767 ± 958	11.4	7340 ± 160	13.4
		SLA-DRA/DRB	anti-SLA-DR	1467 ± 121	2.5	1350 ± 373	2.5
hPBMC	allo-hPBMC[5]	/	/	13380 ± 1122	22.6	11187 ± 1601	20.5

[1] Human PBMCs.
[2] Stimulating cells with or without gene transfection were irradiated at 3000 rads.
[3] The L929 clone transfected with empty vector.
[4] The L929 clone transfected with Bm-DRA/DRB genes.
[5] Allogeneic human PBMCs without gene transfection used as positive control.

Human T cell responses to the cells expressing the SLA-DR molecules of Chinese pig Bm

MHC DR molecule is a heterodimer consisting of α chain and β chain. The two chains are encoded by DRA and DRB genes separately. To express a functional DR molecule of Chinese pigs, the Bm-DRA and Bm-DRB genes were cloned and cotransfected into L929, a mouse fibroblast cell line. Figure 4 shows that the expression of Bm-DR molecule on the cells could be effectively identified by flow cytometry with anti-SLA-DR monoclonal antibody. Functional assay with one-way MLC (mixed leukocyte culture) indicated that only little response of human T cells to empty vector-transfected L929 cells could be detected. While L292 was genetically modified with Bm-DRA/DRB genes, there was a strong xenogeneic reaction, resulting in stimulating indices 11.4 and 13.4 in two independent experiments (Table 3). The data in the Table also show that the reaction could be blocked, at least in part, by an anti-SLA-DR mAb.

Discussion

The three strains of Chinese pigs adopted in our study are not really inbred ones, but they are maintained in a highly isolated condition for a long period in the animal breeding stations. As indicated, for example, two of them are stable enough with an intra-strain similarity coefficient as high as 0.928 and 0.933 (10). It is thus not surprising that only one allele was detected in our study for each DRA and DRB locus of the three strains. It is of course still possible that other DRB alleles might exist and be detected.

MHC DRA genes are of little polymorphism in human. It seems also true in pigs since the SLA-DRA gene sequences are identical not only within three Chinese pig strains but also with NIH minipigs. For SLA-DRB, nevertheless, each Chinese pig strain has its own gene sequence that is different from any reported SLA-DRB alleles. China is rich in natural source of pig strains, so it is hopeful that there would be more pig strains involved in MHC-related immunogenetics study to reveal new class II alleles.

T cells recognize allogeneic or xenogeneic antigens by using either direct or indirect pathway. It was reported that both pathways could be adopted in recognition of SLA antigens by human T cells (7, 8, 18), but probably only indirect pathway is suitable for human T cells' recognition of H-2 antigens (20), a phenomenon consistent with the phylogenic distances detected in our study between species: human *vs.* pig more than human *vs.* mouse (Table 1 and 2). The main criterion to distinguish the two pathways is the origin of involved APCs. In case of direct recognition, donor's leukocytes can work as both stimulators and APCs. For indirect recognition, however, only APCs from recipients are functioned. Table 3 shows that in our study the human T cells responded well to the SLA-DR-expressed L929 cells in an indirect way because only human APCs (B cells and monocytes) were contained in PBMCs. It seems attractive enough to suspect that the substitution of one crucial residue both in swine DRα and DRβ chain might be strongly enough to damage the collaboration between the human CD4 coreceptor and the swine DR molecule, which forces the human CD4-positive T cells to use the indirect pathway to recognize the SLA class II antigens.

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