Novel SLA class I alleles of Chinese pig strains and their significance in xenotransplantation

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ABSTRACT

To lay background for studying rejection mechanisms in xenotransplantation and developing the strategies for intervention, class I genes of swine leukocyte antigens (SLA) of three Chinese pig strains Bm, Gz and Yn were cloned and sequenced. The cDNA of the class I loci P1 and P14 were amplified by RT-PCR and subjected to insert into sequencing vectors. All six allelic sequences we examined, each two for one Chinese strain, are not identical to those reported, which allows these novel sequences receiving their accession numbers AY102467-AY102472 from GenBank. This study further reveals that the homologies of MHC class I genes in their primary structures and the deduced amino acids between Chinese pigs (SLA) and human (HLA-A*0201) are better than those between pigs and mice (H-2Db/H-2Kb). The comparison also indicates that the amino acid residues critical for recognition by human KIRs are altered in the swine class I molecules. The amino acids responsible for binding human CD8 coreceptor are largely conserved although there are two critical residues substituted. A functional test indicated that the human T cells specific for the prokaryotically expressed SLA P1protein could respond quite well in vitro to the class I-positive swine chondrocytes and PBMCs in presence of human APCs. This implies that, due to the substitution of two critical residues, the inaccessibility of human CD8 coreceptor to swine class I molecule might be contributable to the indirect pathway that the human T cells have to use for recognizing the SLA class I xenogeneic antigens.

Key words: *swine leukocyte antigens (SLA), Chinese pig train, xenotransplantation, killer cell inhibitory receptor (KIR), CD8 coreceptor.*

INTRODUCTION

Discordant xeno-grafting using pigs as organ donors have been regarded one of the most hopeful approaches to solve the shortage of organ grafts in clinic transplantation[1, 2]. There are series of complicated rejection responses concerned. Among them, for example, are the hyperactive rejection (HAR), the delayed xeno-grafts rejection (DXR) and the acute cellu-

lar rejection (ACR)[1, 2]. The break-through in overcoming HAR by obtaining the transgenic pig with human DAF gene[3, 4] and by production of α-1, 3-galactosyltransferase knockout pigs by nuclear transfer cloning[5] have push the investigation further towards DXR and ACR. It is thus of importance to elucidate the structures of swine leukocyte antigen (SLA) genes, the pig MHC, from as many strains as possible, since either cytotoxic activity of NK cells in DXR and cellular rejection in ACR all depends on expression of SLA[2, 6, 7].

SLA molecules are encoded by a group of tightly linked genes located on 7p1.1 and 7q1.1 of pig chromosome. The main loci of SLA class I genes are P1 and P14[8, 9]. China is rich in natural resources

of pigs. Many strains are traditionally bred in a highly isolated condition. It is thus valuable to select those from Chinese pig strains with satisfied immunogenetic properties as candidates for xenotranspantation.

Based on the structures of the NIH minipig class I genes, SLA cDNA of three Chinese pig strains were cloned and amplified by RT-PCR. The strains are all endowed with new sequences for class I genes. Some related studies were also performed to functionally confirm the structures and to explore their significances in xenogeneic rejection.

MATERIALS AND METHODS

Pig strains

Three Chinese miniature pig strains used for our studies offered by the Experimental Animal Center, Chinese Academic of Sciences. They are Bama pig (Bm), Guizhou Xiang pig (Gz) and Yunan Banna pig (Yn). Bm and Gz 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 MHC class I cDNA

Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) of the miniature pigs using TRIzol reagent following the manufacturer's protocol (Gibco BRL) and 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 from GenBank database for P1 and P14 of NIH minipigs[12], two pairs of primers were designed and synthesized with software Primer Express 3.0:

P1: up- 5'ATCGAAGCTTATGGGGCCTGGAGCCCTCTTCCTG
 down- 5'CGATCTCGAGTCACACTCTAGGATCCTTGGGTAAGGGAC
P14: up-5'ATCGAAGCTTATGCGGGTCAGAGGCCCTCAAGCCATCCTCATTC
 down- 5'CGATCTCGAGTCACACTCTAGGATCCTTGGGTAAGGGAC

The sites for restriction enzymes Hind III (AAGCTT) and XhoI (CTCGAGT) are inserted and indicated by underlines.

PCR was performed by a 'ouchdown' method[13] with a DNA thermal cycler (Perkin-Elmer 480). Denaturation was conducted at 94°C for 1 min. Annealing was performed at temperatures ranging from 72°C to 63°C, lowing 1°C for each two cycles. The amplification was finished with fifteen cycles at 63°C.

Cloning and Sequencing of SLA class I cDNA

PCR products were purified with QIAquick Gel Extraction (Qiagen, Chatswoth, CA) and inserted into pGEM-T Easy vector (Promega, Madison, WI). The SLA class I cDNA clones were analyzed by restriction mapping as follows: 1 mg of DNA (SLA class I 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. 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. Autoassembler (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 MHC class I cDNA. These sequences were searched against GenBank databases for homology comparison using BLAST in the Genetics Computer Group program package.

Prokaryotic expression of class I SLA P1 gene of Yn pig and purification of its protein

The Yn pig SLA P1 cDNA with no signal peptide-encoded sequence was PCR amplified followed by sequencing to confirm the gene structure. After digestion with restriction enzyme, the subclonal fragment were inserted into expression vectors to construct the recombinant expression vectors pET-42b (+)/sla-p1, which were then induced to express by IPTG as P1-8×his fusion proteins. The detail procedures for purification and identification of the protein are described in a separate paper[23].

Developing the SLA P1-specific T cell lines and determining their responses to class I SLA antigens

Freshly isolated human PBMCs were washed and suspended in RPMI 1640 culture medium (GibcoBRL) supplemented with 10% human AB sera. 0.2 μl of the cells at concentration 1×10^6/ml were seeded into each well on 96-well culture plates (Nunc) together with the SLA P1 protein at final concentration up to 12.5 μg/ml for cultivation at 37°C for 7 days. After stimulating three times with the P1 protein in a period for 21 day, the cells in positive wells were picked up to determine their surface markers and maintained to make sure they are the SLA P1-specific T cell lines. For functional analysis, some of the cell lines were used as responding T cells in a xenogeneic mixed lymphocyte chondrocyte culture with freshly isolated chondrocytes or PBMCs of Yn pig strain as stimulators. Both PBMCs and chondrocytes were irradiated with X-rays at 3000 rads. Lymphoproliferation to SLA class I antigens was determined by ^{3}H-TdR uptake, expressed as mean$\pm$SD of cpm (counts per minute).

RESULTS

Analysis of the SLA class I cDNA from Chinese pig strains

A clear RT-PCR amplified band of P1 or P14 cDNA is seen in electrophoresis on agarose gel (Fig 1A). The sizes of amplified products of P1 and P14

are nearly identical, round 1.1 kb as expected. The figure only shows the results from Bm pig as an example. Similar results were obtained for Gz and Yn pigs (results not presented).

After purification, the PCR products were inserted into pGEM-T Easy vector and digested with Hind III and XhoI, followed by separating on agarose gels. Fig 1B presents the related results of electrophoresis for SLA class I genes of Bm pig, including the P1/P14-cocantained recombinant plasmids (lines b to e) and their products digested with the restriction endonucleases (lines f to i). For the later, there are two bands for the vector and the amplified products (1.1 kb) respectively, indicating a successful ligation of SLA class I cDNA with the sequencing vector. There are similar results for the other two Chinese pig strains (data not included).
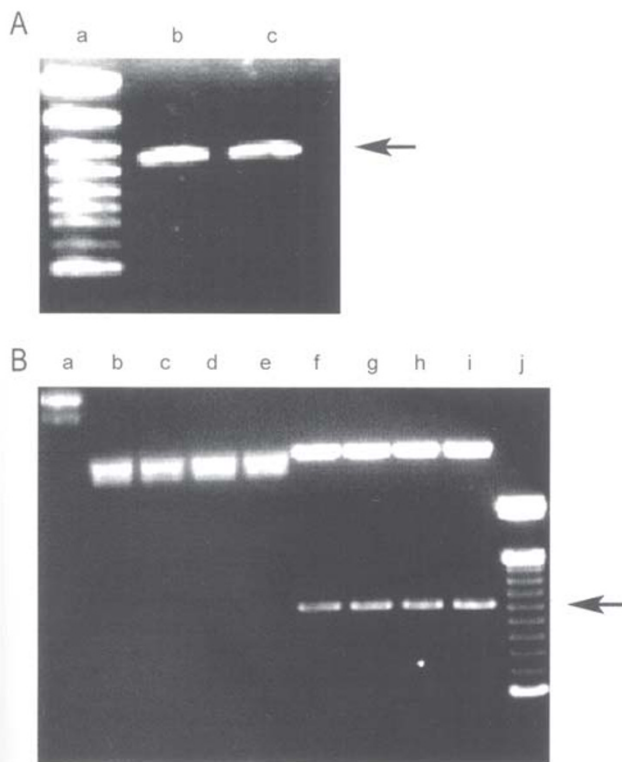
Fig 1. RT-PCR-amplified SLA class I genes of Chinese Bama pig
(**A**) Electrophoresis of cDNA: (a) molecular weight; (b) P1 locus; (c) P14 locus
(**B**) Electrophoresis patterns of the restriction enzyme-digested SLA class I cDNA clones of Chinese Bama pig
(a) l/Hind III+Ecor   (f) P1-pGEM-T-a /Hind III+Xho I
(b) P1-pGEM-T-a   (g) P1-pGEM-T-b /Hind III+Xho I
(c) P1-pGEM-T-b   (h) P14-pGEM-T-a /Hind III+Xho I
(d) P14-pGEM-T-a   (i) P14-pGEM-T-b /Hind III+Xho I
(e) P14-pGEM-T-b   (j) 100 bp marker

SLA class I gene sequences 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 SLA class I cDNA ORF-containing sequences for Chinese pig strains (Fig 2). For each strain, there is only one allele detected either for P1 or P14 locus.

As indicated in Fig 2, for P1 locus we have alleles Bm-P1, Gz-P1 and Yn-P1, respectively. They all consist of 1086 nucleotides. Each encodes for a polypeptide of 361 amino acid residues with a terminal codon. The polypeptide includes signal peptide (aa 1- 21), a1 domain (aa 22 - 111), a2 domain (aa 112 -203), a3 domain (aa 204 - 295) and a membrane/plasma fragment (aa 296 - 361). Homologies among Bm-P1, Gz-P1 and Yn-P1 are 95.1% - 98.7% at nucleotide level and 90.1% - 97.5% at amino acid level.

Similarly, three alleles with 1095 nucleotide encoding for a polypeptide of 364 aa residues were detected for Bm-P14, Gz-P14 and Yn-P14 loci, respectively. The polypeptide also contains signal peptide (aa 1 - 24), a1 domain (aa 25 - 114), a2 domain (aa 115 - 206), a3 domain (aa 207 - 298) and a membrane/plasma fragment (aa 299 - 364). There are nine additional nucleotides at 5' terminal in P14 genes when compared with P1 genes, resulting in different sizes for signal peptides although other domains keep unchanged in nucleotide numbers (Fig 2).

Structural comparison of class I genes of Chinese pig strains with those of NIH minipigs

Setting the ORF-containing sequences of SLA-P1 and -P14 alleles of NIH minipigs as a 'standard', the structures of P1 and P14 alleles from three Chinese pig strains were compared and analyzed at protein level. As shown in Fig 3, the sequence differences are existed not only within P1 molecules (Bm-P1, Gz-P1, Yn-P1) and P14 molecules (Bm-P14, Gz-P14, Yn-P14), but also between Chinese pigs and NIH minipigs. Ranges of the structural discrepancies are similar. Interestingly enough, amino acid substitutions of class I molecules are restricted to a1(22-111) and a2 (112-203) domains, which contain crucial sequences for construction of antigen-binding clefts.

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           1
Bm-P1  ~ ~ ~ ~ ~ ~ ~ ~ A TGGGGCCTGG AGCCCTCTTC CTGCTGCTGT CGGGGACCCT
Gz-P1  ~ ~ ~ ~ ~ ~ ~ ~ A TGGGGCCTGG AGCCCTCTTC CTGCTGCTGT CGGGGACCCT
Yn-P1  ~ ~ ~ ~ ~ ~ ~ ~ A TGGGGCCTGG TCTTCAGCCC CTGCTGCTGT CGGGGACCCT
Bm-P14 ATGCGGGTCA GAGGCCCTCA AGCCATCCTC ATTCTGCTGT CGGGGGCCCT
Gz-P14 ATGCGGGTCA GAGGCCCTCA AGCCATCCTC ATTCTGCTGT CGGGGGCCCT
Yn-P14 ATGCGGGTCA GAGGCCCTCA AGCCATCCTC ATTCTGCTGT CGGGGGCCCT
           51
Bm-P1  GGCCCTGACC GGGACCCAGG CGGGTCCCCA CTCCCTGAGC TATTTCTACA
Gz-P1  GGCCCTGACC GGGACCCAGG CGGGTCCCCA CTCCCTGAGC TATTTCTACA
Yn-P1  GGCCCTGACC GGGACCCAGG CGGGTCCCCA CTCCCTGAGC TATTTCTACA
Bm-P14 GGCCCTGACC GGGACCTGGG CGGGTCCCCA CTCCCTGAGG TATTTCCACA
Gz-P14 GGCCCTGACC GGGACCCAGG CGGGTCCCCA CTCCCTGAGC TATTTCTACA
Yn-P14 GGCCCTGACC GGGACCCAGG CGGGTCCCCA CTCCCTGAGG TATTTCTACA
           101
Bm-P1  CCGCCGTGTC CCGGCCCGAC CGCGGGGACT CTCGCTTCAT CGCCGTCGGC
Gz-P1  CCGCCGTGTC CCGGCCCGAC CGCGGGGACT CTCGCTTCAT CGCCGTCGGC
Yn-P1  CCGCCGTGTC CCGGCCCGAC CGCGGGGACT CCCGTTTCAT CGAAGTCGGC
Bm-P14 CCGCCGTGTC CCGGCCCGAC CGCGGGGACT CCCGCTTCCT CACCGTCGGC
Gz-P14 CCGCCGTGTC CCGGCCCGAC CGCGGGGAGC CCCGCTTCAT CGCCGTCGGC
Yn-P14 CCGCCGTGTC CCGGCCCGAC CGCGGGGACT CCCGCTTCCT CACCGTCGGC
           151
Bm-P1  TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAACTACG CCCCGAATCC
Gz-P1  TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAACTACG CCCCGAATCC
Yn-P1  TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAGCGACG CCCCCAATCC
Bm-P14 TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAGCGACG CCCCGAATCC
Gz-P14 TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAGCGACG CCCCCAATCC
Yn-P14 TACGTGGACG ACACGCAGTT CGTGCGGTTC GACAGCGACG CCCCGAATCC
           201
Bm-P1  GCGGATGGAG CCTCGGGTGC CGTGGATACA GCAGGAGGGG CAGGAGTATT
Gz-P1  GCGGATGGAG CCTCGGGTGC CGTGGATACA GCAGGAGGGG CAGGAGTATT
Yn-P1  GCGGATGGAG CCGCGGGCGC CGTGGATACA GCAGGAGGGG CAGGAGTATT
Bm-P14 GAGGAAAGAG CCGCGGGCGC CGTGGATAGA GCAGGAGGGG CAGGACTATT
Gz-P14 GCGGATGGAG CCTCGGGTGC CGTGGATACA GCAGGAGGGG CAGGAGTATT
Yn-P14 GAGGGAGGAG CCGCGGGCGC CGTGGATCGA ACAGGAGGGG CAGGACTATT
           251
Bm-P1  GGGATCGGGA GACGCGGAAT GTCAAGGAAA CCGCACAGAC TTACGGAGTG
Gz-P1  GGGATCGGGA GACGCGGAAT GTCAAGGAAA CCGCACAGAC TTACGGAGTG
Yn-P1  GGGATGAGGA GACGCGGCGT GTCATGGGCA GCGCACAGAT TTACCGAGTG
Bm-P14 GGGATCGGGA GACACAGATC AGCAAGGACA ACGCAGAGAT TAACCGAGTG
Gz-P14 GGGATCGGGA GACGCAGATA CAAAGGGACA CCTCACAGAC TTACGGAGTG
Yn-P14 GGGATCGGGA GACACAGATC AGCAAGGAAA CTGCACAGAC TTACCGAGTG
           301
Bm-P1  GGCCTGAACA CCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
Gz-P1  GGCCTGAACA CCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
Yn-P1  AACCTGAACA CCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
Bm-P14 GGCCTGAACA CCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
Gz-P14 GGCCTGAACA CCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
Yn-P14 GGCCTGAAGA TCCTGCGCGG CTACTACAAC CAGAGCGAGG CCGGGTCTCA
           351
Bm-P1  CACCCTCCAG AGCATGTACG GCTGCTACTT GGGACCAGAC GGGCTCCTCC
Gz-P1  CACCCTTCAG AGCATGTACG GCTGCTACTT GGGACCAGAC GGGCTCCTTC
Yn-P1  CACCCTCCAG TGGATGTACG GCTGCGACGT GGGACCAGAC GGGCTCTTCC
Bm-P14 CACCCTCCAG AGCATGTACG GCTGCGACGT GGGACCAGAC GGGCTCTTCC
Gz-P14 CACCATCCAG AGCATGTACG GCTGCTACTT GGGACCAGAC GGGCTCCTCC
Yn-P14 CACCTACCAG TGGATGTACG GCTGCTACTT GGGACCAGAC GGGCTCTTCC
           401                    .
Bm-P1  TCCACGGGTA CAGACAGGAC GCCTACGACG GCGCCGATTA CATCGCCCTG
Gz-P1  TCCACGGGTA CAGACAGGAC GCCTACGACG GCGCCGATTA CATCGCCCTG
Yn-P1  TCCGCGGGTA CAGTCAGGAC GCCTACGACG GCGCCGATTA CATCGCCCTG
Bm-P14 TCCGCGGGTA CAGTCAGGAC GCCTACGACG GCGCCGATTA CATTGCCCTG
Gz-P14 TCCGCGGGTA CAGTCAGGAC GCCTACGACG GCGCCGATTA CGTCGCCCTG
Yn-P14 TCCGCGGGTA CAGTCAGTAT GGCTACGACG GCGCCGATTA CATCGCCCTG
           451
Bm-P1  AACGAGGACC TGCGCTCCTG GACCGCGGCG GACATGGCGG CTCAGAACAC
Gz-P1  AACGAGGACC TGCGCTCCTG GACCGCGGCG GACATGGCGG CTCAGATCAC
Yn-P1  AACGAGGACC TGCGCTCCTG GACCGCGGCG GACACGGCGG CTCAGATCAC
Bm-P14 AACGAGGACC TGCGCTTCTG GACCGCGGCG GACATGGCGG TTCAGATCAC
Gz-P14 AACGAGGACC TGCGCTCCTG GACCGCGGCG GACATGGCGG CTCAGATCTC
Yn-P14 AACGAGGACC TGCGCTCCTG GACCGCGGCG GACACGGCGG CTCAGATCAC
           501
Bm-P1  CAAGTGCAAG TGGGAGGCGG CCGATGAGGC GGAACGTAAG AGGAGCTACC
Gz-P1  CAAGCGCAAG TGGGAGGCGG CCGATGAGGC GGAACGTAAG AGGAGCTACC
Yn-P1  CAAGCGCAAG TGGGAGACGG CCAATGTGGC GGAGCAAGAG AAGAGCTACC
Bm-P14 CAAGCGCAAG TGGGAGGCGG CCGATGCGGC GGAGGAGGAG AGGAGCTACC
Gz-P14 CAAGCGCAAG TGGGAGGCGG CCGATGTGGC GGACAATTG AGGAGCTACC
Yn-P14 CAAGCGCAAG TGGGAGGCGG CCGATGAGGC GGAGCAAATG AGGAGCTACC
           551
Bm-P1  TGCAGGGACT GGGGGTGGAG GCGCTTCCGA AATACCTGGA GAAGGGGAAG
Gz-P1  TGCAGGGACT GTGTGTGGAG TCGCTCCGCA GATACCTGGA GATGGGGAAG
Yn-P1  TGCAGGGCCT GTGTGTGGAG TGGCTCCAGA AATACCTGGA GATGGGGAAG
Bm-P14 TGCAGGGCAC GTGTGTGGAG TGGCTCCAGA GATACCTGGA GATGGGGAAG
Gz-P14 TGCAGGGCAC GTGTGTGGAG TGGCTCCAGA AATACCTGGA GATGGGGAAG
Yn-P14 TGCAGGGCCT GTGTGTGGAG TGGCTCCAGA AATACCTGGA GATGGGGAAG
           601
Bm-P1  GACACGCTGC AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
Gz-P1  GACACGCTGC AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
Yn-P1  GACACGCTGC AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
Bm-P14 GACACCTGCA AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
Gz-P14 GACACGCTGC AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
Yn-P14 GACACGCTGC AGCGCGCAGA GCCTCCAAAG ACACATGTGA CCCGCCACCC
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         651
Bm-P1   C A G C T C T G A C  C T C G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
Gz-P1   C A G C T C T G A C  C T C G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
Yn-P1   C A G C T C T G A C  C T C G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
Bm-P14  C A G C T C T G A C  C T G G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
Gz-P14  C A G C T C T G A C  C T C G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
Yn-P14  C A G C T C T G A C  C T G G G G G T C A  C C T T G A G G T G  C T G G G C C C T G  G G C T T C T A C C
         701
0Bm-P1  C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G G G  A G G G C C A G G A  C C A G A G C C A G
Gz-P1   C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G G G  A G G G C C A G G A  C C A G A G C C A G
Yn-P1   C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G G G  A G G G C C A G G A  C C A G A G C C A G
Bm-P14  C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G G G  A G G G C C A G G A  C C A G A G C C A G
Gz-P14  C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G G G  A G G G C C A G G A  C C A G A G C C A G
Yn-P14  C T A A G G A G A T  C T C C C T G A C C  T G G C A G C G C G  A G G G C C A G G A  C C A G A C C C A G
         751
Bm-P1   G A C A T G G A G C  T G G T G G A G A C  C A G G C C C T C A  G G G G A T G G G A  C C T T C C A G A A
Gz-P1   G A C A T G G A G C  T G G T G G A G A C  C A G G C C C T C A  G G G G A T G G G A  C C T T C C A G A A
Yn-P1   G A C A T G G A G C  T G G T G G A G A C  C A G G C C C T C A  G G G G A T G G G A  C C T T C C A G A A
Bm-P14  G A C A T G G A G C  T G G T G G A G A C  C A G G C C C T C A  G G G G A T G G G A  C C T T C C A G A A
Gz-P14  G A C A T G G A G C  T G G T G G A G A C  C A G G C C C T C A  G G G G A T G G G A  C C T T C C A G A A
Yn-P14  G A C A T G G A G C  T C G T G G A G A C  C A G G C C T T C A  G G G G A T G G G A  C C T T C C A G A A
         801
Bm-P1   G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
Gz-P1   G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
Yn-P1   G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
Bm-P14  G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
Gz-P14  G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
Yn-P14  G T G G G C G G C C  C T G G T G G T G C  C T C C T G G A G A  G G A G C A G A G C  T A C A C C T G C C
         851
Bm-P1   A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C A T G A G  A T G G G A C C C T
Gz-P1   A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C C T G A G  A T G G G A C C C T
Yn-P1   A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C C T G A G  A T G G G A C C C T
Bm-P14  A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C C T G A G  A T G G G A C C C T
Gz-P14  A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C C T G A G  A T G G G A C C C T
Yn-P14  A T G T G C A G C A  C G A G G G C C T G  C A G G A G C C C C  T C A C C C T G A G  A T G G G A A C C T
         901
Bm-P1   G C T C A G C C C C  C C G T C C C C A T  C G T G G G C A T C  A T T G T T G G C C  T G G T T C T G G T
Gz-P1   G C T C A G C C C C  C C G T C C C C A T  G G T G G G C A T C  A T T G T T G G C C  T G G T T C T G G T
Yn-P1   G C T C A G C C C C  C C G T C C C C A T  C G T G G G C A T C  A T T G T T G G C C  T G G T T C T C G T
Bm-P14  G C T C A G C C C C  C C G T C C C C A T  G G T G G G C A T C  A T T G T T G G C C  T G G T T C T G G T
Gz-P14  C C T C A G C C C C  C C G T C C C C A T  C G T G G G C A T C  A T T G T T G G C C  T G G T T C T T G T
Yn-P14  C C T C A G C C C C  C C G T C C C C A T  C G T G G G C A T C  A T T G T T G G C C  T G G T T C T T G T
         951
Bm-P1   C C T G G T C G C T  G G A G C C A T G G  T G G C T G G A G T  T G T G A T C T G G  A G G A A G A C G C
Gz-P1   C C T G G T C G C T  G G A G C C A T G G  T G G C T G G A G T  T G T G A T C T G G  A G G A A G A C G C
Yn-P1   C C T G G T C G C T  G G A G C C G T G G  T G G C T G G A G T  T G T G A T C T G G  A G G A A G A A G C
Bm-P14  C C T G G T C G C T  G G A G C C G T G G  T G G C T G G A G T  T G T G A T C T G G  A G G A A G A A G C
Gz-P14  C C T G G T C G C T  G G A G C C G A T G G  T G G C T G G G G T  T G T G A T C T G G  A G G A A G A A G C
Yn-P14  C C T G G T C A C T  G G A G C C A T G G  T G G C T G G A G T  T G T G A T C T G G  A G G A A G A A G C
         1001
Bm-P1    G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  C A G T G A C A G T
----Gz-P1 G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  C A G T G A C A G T
Yn-P1    G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  C A G T G A C A G T
Bm-P14   G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  G A G T G A C A G T
Gz-P14   G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  C A G T G A C A G T
Yn-P14   G C T C A G G T G A  A A A A G G A G G G  A G C T A C A C T C  A G G C T G C A G G  C A G T G A C A G T
         1051
Bm-P1   G A C C A G G G C T  C C G A T G T G T C  C C T T A C C A A G  G A T C C T A G A G  T G T G A
Gz-P1   G A C C A G G G C T  C C G A T G T G T C  C C T T A C C A A G  G A T C C T A G A G  T G T G A
Yn-P1   G A C C A G G G C T  C C G A T G T G T C  C C T T A C C A A G  G A T C C T A G A G  T G T G A
Bm-P14  G C C C A G G G C T • C C G A T G T G T C  C C T T A C C A A G  G A T C C T A G A G  T G T G A
Gz-P14  G C C C A G G G C T  C C A A T G T G T C  C C T T A A C A A G  G A T C C T A G A G  T G T G A
Yn-P14  G C C C A G G G C T  C C G A T G T G T C  C C T T A C C A A G  G A T C C T A G A G  T G T G A
```

Fig 2. Heavy chain nucleotide sequences of SLA class I P1 and P14 alleles of three Chinese pig strains

Tab 1. A comparison of MHC class I heavy chains in terms of amino acid homology among the Chinese swine strains, mouse and human

A	Bm-P1	Gz-P1	Yn-P1	SLA-P1[a]	SLA-P1[c]	SLA-P1[d]	A*0201[#]	H-2D[b]	H-2K[b]
Bm-P1		97.5	90.1	92.5	86.4	97.5	70.0	58.5	60.3
Gz-P1	97.5		90.9	94.7	88.1	99.5	70.8	60.2	61.7
Yn-P1	90.1	90.9		89.2	92.0	91.1	73.1	61.6	62.6
B	Bm-P14	Gz-P14	Yn-P14	SLA-P14[a]	SLA-P14[c]	SLA-P14[d]	A*0201[#]	H-2D[b]	H-2K[b]
Bm-P14		89.3	91.0	90.7	90.4	89.6	71.4	60.8	63.1
Gz-P14	89.3		91.2	89.3	94.0	91.2	71.6	61.1	62.3
Yn-P14	91.0	91.2		92.6	91.2	89.8	71.9	61.6	62.9

[#] A*0201 stands for HLA-A*0201, a class I allele with the highest frequency in human populations.

```
        1
SLA-a   - - - M G P G A L F   L L L S G T L A L T   G T Q A G P H S L S   Y F Y T A V S R P D   R G E P P F I A V G
SLA-c   - . . . . . . . . .   . . . . . . . . . .   - - R E - - - - R   - D - . - . . - - .   - R K - R - - S - -
SLA-d   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - - D S R - - - - -
Bm-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - - D S R - - - - -
Gz-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - - D S R - - - - -
Yn-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - - - - - - R - - E - -
A*0201  M A V - A - R T - V   - - - - A - - - -   Q T W - - S - - M R   - - F - S - - - G   - - - - - R - - - - -
        51
SLA-a   Y V D D T Q F V R F   D N Y A P N P R M E   P R V P W I Q Q E G   Q D Y W D E E T R K   V K D N A Q T L R V
SLA-c   - . . . . . . . . .   - - S D - - - - - -   - . . A . . . . .   - K - - - - - Q N - A M G S - - - F - -
SLA-d   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - E - - R - - - N - - E T - - - Y - -
Bm-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - E - - R - - - N - - E T - - - Y G -
Gz-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   - E - - R - - - N - - E T - - - Y G -
Yn-P1   - . . . . . . . . .   - - S D - - - - - -   - . . A . . . . .   - E - - - - - R - - M G S - - I Y G -
A*0201  - . . . . . . . . .   - S D - A S Q - - -   - . . A - - E - - -   P E - - - G - - - A H S - - - H - -
        101
SLA-a   G L N T L R G Y Y N -   Q S E A G S H T L Q   S M F G C Y L G P D   G L L L H G Y R Q D   A Y D G A D Y I A L
SLA-c   N - K N - . - . . .   . . . . . . . . . .   - - Y - - D V - -   - - F - R - - H - -   - . . . . . . . . .
SLA-d   - . . . . . . . . .   . . . . . . . . . .   - - Y - . . . . .   . . . . . . . . . .   . . . . . . . . . .
Bm-P1   - . . . . . . . . .   . . . . . . . . . .   - - Y - . . . . .   . . . . . . . . . .   . . . . . . . . . .
Gz-P1   - . . . . . . . . .   . . . . . . . . . .   - - Y - . . . . .   . . . . . . . . . .   . . . . . . . . . .
Yn-P1   N - . . . . . . . .   . . . . . . . . . .   W - Y - - D V - -   - - F - R - - S - -   - . . . . . . . . .
A*0201  D - G - . . . . . .   . . . . . . . . . .   - V - R - Y - - D V - S -   W R F - R - - H - Y   - - - - K - - - - -
        151
SLA-a   N E D L R S W T A A   D M A A Q I S K R K   W E A A D E A E R M   R S Y L Q G R C V E   G L R R Y L Q M G K
SLA-c   - . . . . . . . . .   - T - - - - A - -   - . . . . . . - Q W   - - - - E - A - - -   W - - Q K - E - - -
SLA-d   - . . . . . . . . .   - - - T - . . . .   - . . . . . . - R   - . . . . . - L - - -   S - - - - - - E - - -
Bm-P1   - . . . . . . . . .   - - - - N - - T - C   - . . . . . . - K   - . . . . . - L - - -   A - P K - - - - E K - -
Gz-P1   - . . . . . . . . .   - - - - T - . . .   - . . . . . . - K   - . . . . . - L - - -   S - - - - - - E - - -
Yn-P1   - . . . . . . . . .   - T - - - - T - - -   - - T - N V - - Q E   K - - - - L - - -   W - Q K - E - - -
A*0201  K - . . . . . . . .   - - M - - - - - T - H   - - - - H V - - Q L   - A - E - T - - -   W - - - - - E N -
        201
SLA-a   D T L Q R A D P P K   T H V T R H P S S D   L G V T L R C W A L   G F Y P K E I S L T   W Q R E G Q D Q S Q
SLA-c   - . . . . . E - . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
SLA-d   - . . . . . E - . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Bm-P1   - . . . . . E - . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Gz-P1   - . . . . . E - . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Yn-P1   - . . . . . E - . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
A*0201  E - - - - - - A - - -   - M - H - A V - H E A   - - - - - - - -   S - - - A - - T - - - - -   D - E - T -
        251
SLA-a   D M E L V E T R P S   G D G T F Q K W A A   L W P P G E E Q S   Y T C H V Q H E G L   Q E P L T L R W D P
SLA-c   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . .   . . . . . . . . . .   . . . . . . . - D -
SLA-d   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . .   . . . . . . . . . .   . . . . . . . - D -
Bm-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . .   . . . . . . . . . .   . . . . . . . - D -
Gz-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . .   . . . . . . . . . .   . . . . . . . - D -
Yn-P1   - . . . . . . . . .   . . . . . . . . . .   . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
A*0201  - T - - - - - - A -   - . . . . . . . . .   V - - - S - Q - - -   - . . . . . . . . .   P K - - - - - - -
        301
SLA-a   / A Q P P V P M V G   I T V G L V L / / V   L V A G A M V A G V   V I W R K T R S G E   K G G S Y T Q A A G
SLA-c   - . . . . . . . . .   - I - . . . . . . .   - - - - A - - A - -   - . . . . . . . . .   . . . . . . . . . .
SLA-d   - . . . . . . . . .   - I - . . . . . . .   - - - A V - A - -   - . . . . . . . . .   . . . . . . . . . .
Bm-P1   - A - - - - - M - - -   - I - . . . . . . .   - - - A V - A - -   - . . . . . . . . .   . . . . . . . . . .
Gz-P1   - . . . . . . . . .   - I - . . . . . . .   - - - - A - - A - -   - . . . . . . . . .   . . . . . . . . . .
Yn-P1   - . . . . . . . . .   - I - . . . . . . .   - - - - A - - A - -   - . . . . . . . . .   . . . . . . . . . .
A*0201  S - - - - - - - - -   - I A - - - - F G A   V I T - A V - A - A   - M - R - S - - D R   - - - - - - S - - - -
        351                           .              361
SLA-a   S D S A Q G S D V S L   T   K   D   P   R   V
SLA-c   - . . . . . . . . .   -   .   .   .   - .
SLA-d   - . . . . . . . . .   .   .   .   .   . .
Bm-P1   - . . . . . . . - -   -   .   .   .   . .
Gz-P1   - . . . . . . M - -   N   .   -   -   . .
Yn-P1   - . . . . . . . . .   .   .   .   .   . .
A*0201  - - - - - - - - - -   -   A   C   R   - -
```
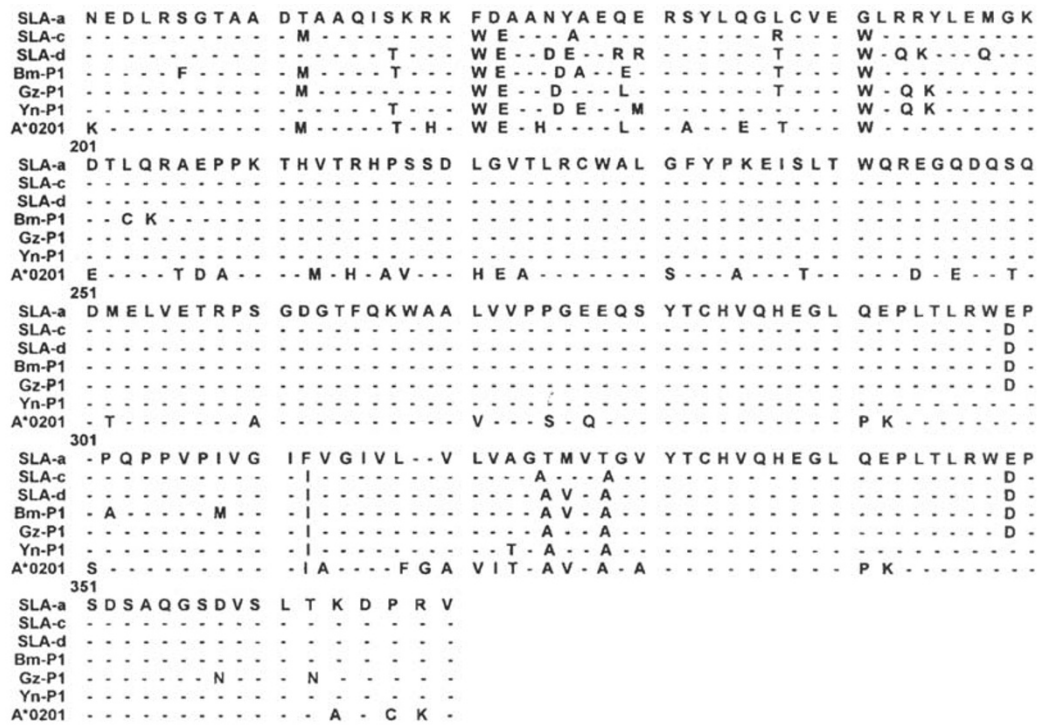
```
        1
SLA-a   M R V R G P Q A I L   I L L S G A L A L T   G T R A G P H S L R   Y F D T A Y S R P D   R G D S R F L T V G
SLA-c   - . . . . . . . . .   . . . . . . . . . .   - . . . . . - : - - S   - Y - . . . . . . .   L - . . . . . I A -
SLA-d   - . . . . . . . . .   . . . . . . . . . .   - - Q - . . . . . .   - . . . . . . . . .   - - - E P - - I E -
Bm-P1   - . . . . . . . . .   . . . . . . . . . .   - - W - . . . . . .   - . . . . . . . . .   - . . . . . . . . .
Gz-P1   - . . . . . . . . .   . . . . . . . . . .   - - Q - . . . . . - S   - - Y - . . . . .   - - E P - - I A -
Yn-P1   - . . . . . . . . .   . . . . . . . . . .   - - Q - . . . . . .   - Y - . . . . . . .   . . . . . . . . . .
A*0201  - A - M A - - T L V   L - . . . . . . . .   Q - W - - S - - M -   - - F - S - - - -   G - - E P - - I A -
        51
SLA-a   Y V D D T Q F V R F   D S D A P D P R E E   P R A P W I Q Q E G   Q D Y W D R E T Q I   S K E T A Q T Y R V
SLA-c   - . . . . . . . . .   - . . . . . . - M -   - . . . . . E K - - E   - . . . . . . . .   Q R D - S - - - - -
SLA-d   - . . . . . . . . .   - . . . . . . - M -   - . . . . . . . .   - E - - - N - R N   A M G N - - I - - G
Bm-P1   - . . . . . . . . .   - . . . . . . - K -   - . . . . E - . .   - . . . . . . . .   - - D N - E I N - -
Gz-P1   - . . . . . . . . .   - . . . . . . - M -   - . . . . . . . .   - E - . . . . . .   Q R D - S - - - - -
Yn-P1   - . . . . . . . . .   - . . . . . . . . .   - . . . . E - . .   - . . . . . . . .   . . . . . . . . . .
A*0201  - . . . . . . . . .   - - - - A S Q - M -   - . . . . . E - - -   P E - - - G - - R K   K - A H S - H - -
        101
SLA-a   D L N T L R S Y Y N   Q S E A G S H T L Q   S M Y G C D V G P D   G L F L R G Y S Q F   G Y D G A D Y L A L
SLA-c   - - K - - G - . . .   . . . . . . . . . .   - . . . . Y L - - -   - - L - - - - R - -   A - . . . . . . . .
SLA-d   - N - R - A L G - - -   - . . . . . . - I   - I - . . . . . . .   - - L - - - - - D A   - . . . . . . I - -
Bm-P1   G - . . . . - G - -   - . . . . . . . . .   - I - . . . . . . .   - . . . . . . D A   - . . . . . . I - -
Gz-P1   G - . . . . . G - -   - . . . . . . - I   - . . . . Y L - - -   - - L - . . . . D A   - . . . . . . V - -
Yn-P1   G - K I - - G - - -   - . . . . . . - Y   W - . . . Y L - - -   - . . . . . . - Y   A - . . . . . . . .
A*0201  - - G - - - - G - - -   - . . . . . . - V   - R - . . . . . . -   S - W R - - - - - H - Y   A - - - K - I - -
        151
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SLA-a   N E D L R S G T A A   D T A A Q I S K R K   F D A A N Y A E Q E   R S Y L Q G L C V E   G L R R Y L E M G K
SLA-c   . . . . . . . . . .   . . M . . . . . . .   W E . . . A . . . .   . . . . . . R . . .   W . . . . . . . . .
SLA-d   . . . . . . . . . .   . . . . . T . . .     W E . . D E . . R R   . . . . . . T . . .   W . Q K . . . Q . . .
Bm-P1   . . . . . F . . . .   . . M . . . . T . . . W E . . D A . . E .   . . . . . . T . . .   W . . . . . . . .
Gz-P1   . . . . . . . . . .   . . M . . . . . . .   W E . . D . . . L .   . . . . . . T . . .   W . Q K . . . . . .
Yn-P1   . . . . . . . . . .   . . . . . T . . .     W E . . D E . . M .   . . . . . . T . . .   W . Q K . . . . . .
A*0201  K . . . . . . . . .   . . M . . . . . T . H W E . H . . . L .     . A . . E . T . . .   W . . . . . . . . .
        201
SLA-a   D T L Q R A E P P K   T H V T R H P S S D   L G V T L R C W A L   G F Y P K E I S L T   W Q R E G Q D Q S Q
SLA-c   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
SLA-d   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Bm-P1   . . C K . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Gz-P1   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
Yn-P1   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
A*0201  E . . . . T D A . .   . . M . H . A V . . . H E A . . . . . . .   S . . . A . . . T . .   . . . . D . E . . T .
        251
SLA-a   D M E L V E T R P S   G D G T F Q K W A A   L V V P P G E E Q S   Y T C H V Q H E G L   Q E P L T L R W E P
SLA-c   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . D .
SLA-d   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . D .
Bm-P1   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . D .
Gz-P1   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . D .
Yn-P1   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
A*0201  . T . . . . . . A .   . . . . . . . . . .   V . . . S . Q . . .   . . . . . . . . . .   P K . . . . . . . .
        301
SLA-a   . P Q P P V P I V G   I F V G I V L . . V   L V A G T M V T G V   Y T C H V Q H E G L   Q E P L T L R W E P
SLA-c   . . . . . . . . . .   . I . . . . . . . .   . . . A . . . A . .   . . . . . . . . . .   . . . . . . . . D .
SLA-d   . . . . . . . . . .   . I . . . . . . . .   . . . . A V . A . .   . . . . . . . . . .   . . . . . . . . D .
Bm-P1   . A . . . . M . .     . I . . . . . . . .   . . . . A V . A . .   . . . . . . . . . .   . . . . . . . . D .
Gz-P1   . . . . . . . . . .   . I . . . . . . . .   . . . . A . . A . .   . . . . . . . . . .   . . . . . . . . D .
Yn-P1   . . . . . . . . . .   . I . . . . . . . .   . . . T . A . A . .   . . . . . . . . . .   . . . . . . . . . .
A*0201  S . . . . . . . . .   . I A . . . . F G A   V I T . A V . A . A   . . . . . . . . . .   P K . . . . . . . .
        351
SLA-a   S D S A Q G S D V S   L   T   K   D   P   R   V
SLA-c   . . . . . . . . . .   .   .   .   .   .   .   .
SLA-d   . . . . . . . . . .   .   .   .   .   .   .   .
Bm-P1   . . . . . . . . . .   .   .   .   .   .   .   .
Gz-P1   . . . . . . . N . .   .   N   .   .   .   .   .
Yn-P1   . . . . . . . . . .   .   .   .   .   .   .   .
A*0201  . . . . . . . . . .   .   .   A   .   C   K   .
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Fig 3. Predicted amino acid sequences of SLA class 1 P1 (a) and P14 (b) allelic molecules of three Chinese pig strains

Tab 2. A structure comparison of the human KIR-recognized ligands of class I molecules among HLA and correspondent SLA of the Chinese pig strains

	NKTA-1				NKTA-2				NKTA-3						
	77	78	79	80	77	78	79	80	77	78	79	80	81	82	83
HLA class I molecule*	N	L	R	*K* #	*S*	L	R	*N*	N	L	R	*I*	A	L	R
Bm-P1	G	-	N	*T*	G	-	N	*T*	G	-	N	*T*	L	R	G
Gz-P1	G	-	N	*T*	G	-	N	*T*	G	-	N	*T*	L	R	G
Yn-P1	-	-	N	*T*	N	-	N	*T*	N	-	N	*T*	L	R	G
Bm-P14	G	-	N	*T*	G	-	N	*T*	G	-	N	*T*	L	R	G
Gz-P14	G	-	N	*T*	G	-	N	*T*	G	-	N	*T*	L	R	G
Yn-P14	G	-	L	*T*	G	-	K	*T*	G	-	K	*T*	L	R	G

Three KIRs (NKTA-1 to NKTA-3) use ligands (aa sequences from 77-80) with different residues, depending on different class I allelic molecules. NKTA-1 recognizes those from HLA-Cw4, 5, 6; NKTA-2 recognizes those from HLA-Cw1, 3, 7, 8 and NKTA-3 recognizes those from HLA-B 1501, 5801, 2705. An aa sequence from 77-83 is given for B* 5801 on the right columns of the Tab.

\# Dark Italic alphabet stands for a critical aa position in ligand by KIR recognition.

- Indicates that the aa residue is identical to that in HLA class I molecule.

An effort of searching identical alleles from other published SLA class I sequences was not successful (data not shown). A further homology comparison with GenBank databases was performed by using BLAST in the Genetics Computer Group Program package. Our six SLA class I alleles could thus be assigned as novel ones with GenBank accession numbers AY102467 - AY102472.

There are much more discrepancies identified when compared our six SLA alleles with HLA-A*0201, a human MHC class I allele at the highest frequencies both in Chinese and Caucasoid populations. As indicated in Fig 3, the differences not only distribute in the a1 and a2 domains, but also in other part of the molecules, including a3 and the framework. The fact that there are more discrepancies inter-species might account for the stronger immune responses of human T cells to swine MHC antigens than to allogeneic ones.

Tab 1 gives a summary of overall comparison for homologies of amino acid sequences between different kinds of MHC class I allelic molecules when evaluated with GeneDoc software and GCG package. Taking three allelic molecules of P1 locus as an example (Tab 1A), the homologies between Chinese pigs are from 90.1% to 97.5%, in a comparable range from 88.1% to 99.5% for the three alleles of NIH minipigs (SLA-P1a, SLA-P1c and SLA-P1d). When comparing class I molecules of Chinese pigs with other species, however, the inter-species homologies decrease to 70.0% -73.1% for HLA-A*0102, and 58.5%-62.6% for H-2Db and H-2Kb, two mouse MHC class I allelic molecules. Similar situations are found for the alleles of P14 locus (Tab 1B).

The human KIR-recognized ligand sequences in the class I molecules of the Chinese pigs

The killing activities of NK cells are regulated by inhibitory signals from the inhibitory receptor KIR in conjugation with its ligand, the MHC class I molecules on target cells. In case of xenotransplantation, especially during the DXR stage, the ligands offered for recognition by human KIRs are SLA class I molecules expressed on swine endothelium of blood vessel. The failure of the human KIR to ligate to the SLA class I molecules may result in activation of NK cells, leading a damage to the xenograft's endothelium as shown in certain pigs[6, 14]. It is thus of importance to determine the relevant structures in class I molecules of Chinese pig strains. Two residues at position 77 and 80 in KIR's ligand have been recognized crucial for recognition by three kinds of human KIRs, NKTA-1, NKTA-2 and NKTA-3[15-17]. As indicated in Tab 2, for the NKTA-1-related ligand, the two residues are neally all changed in three Chinese pig strains as N 77G and K 80T. Similar situations can be seen for the ligands supposed to be ligated by NKTA-2 and NKTA-3. This analysis implies that, due to replacement of the residues essential for binding with human KIRs in class I molecules of the Chinese pigs, the human NK cells are susceptible to be activated to kill the endothelial cells of donor's blood vessel when the pig organ is xeno-grafted into human recipients.

The sequences of SLA class I that correspond to the HLA counterparts recognized by human CD8 coreceptor

In the acute cellular rejection (ACR) of xeno-grafting, the recognition of xeno-antigens by recipient T cells may dependent on direct or indirect pathway. Which of them is predominant usually depends on the accessibility of accessory molecules between two species especially the receptors on human T cells and the ligand molecules on SLA-expressed cells[7, 19]. In this consideration, the accessibility for human CD8 coreceptors and the SLA molecules on donor endothelium of blood vessel cannot be neglected [20]. The key residues on SLA class I molecule is located in a3 domain, a segment with no polymorphism [21]. To compare the critical sequences of the a3 domain in human (HLA-A*0201) and in the Chinese pig stains, six amino acid residues from 223 to 228 were checked. As indicated in Tab 3, only two of six residues are substituted as T225S and T228M, respectively. In contrast, if the corresponding structures are compared between human (HLA-A*0201) and mouse (H-2Db/H-2Kb), four of six residues show discrepancies (Tab 3), indicating that there is a better accessibility for the coreceptor-ligand conjugation of human with swine than with mouse.

Tab 3. A comparison of the CD8-binding sequences of class I molecules among human, mouse and the Chinese pig strains

| | 2 | 2 | 3 | 2 | 2 | 4 |
| | 2 | 2 | 5 | 2 | 2 | 6 |
	2	2	7	2	2	8
A*0201	D	Q	T	Q	D	T
Bm-P1	-	-	S	-	-	M
Gz-P1	-	-	S	-	-	M
Yn-P1	-	-	S	-	-	M
Bm-P14	-	-	S	-	-	M
Gz-P14	-	-	S	-	-	M
Yn-P14	-	-	S	-	-	M
H-2D^b	E	L	T	-	-	M
H-2K^b	E	L	I	-	-	M

The SLA-P1-specific T cells recognize the SLA class I-expressed chondrocytes in an indirect pathway

To determine the immunogenecity of SLA molecules of Chines pig strains, a P1 protein of Yn pig class I gene was cloned and expressed from the Yn-PI

gene through a prokaryotic expression system. After human PBMCs were stimulated three times in 96-well cell culture plates by our purified Yn-P1 protein at different concentrations, the T cell clones capable of proliferating significantly to Yn-P1 (SI $\geq$ 3) were picked up and subjected to a functional test to determine their responses to natural SLA class I antigens in a xenogeneic mixed lymphocyte chondrocyte culture. The stimulating cells are the SLA class I but not class II-expressed chondrocytes (ChCs) that freshly isolated from same Yn pig donor.

Swine chondrocytes can constitutively express class I but not class II antigens, which were confirmed by our experiments (data not shown). This kind of cells was used as class I-positive stimulating cells to test the reactions of the class I-sensitized T cells. As shown in Tab 4, the Yn-P1-specific T cells responded quite well to the class 1-expressed chondrocytes of Yn pig in presence of APCs (irradiated human PBMCs). The stimulating indices (SI) are 3.2 and 3.1 respectively in two independent experiments. When the chondrocytes were substituted by PBMCs from same pig donors, there appeared a stronger lymphoproliferation (SI = 5.8), suggesting a more effective expression of class I molecules on the swine PBMCs. It is interesting to note, however, that if the swine PBMCs are cocultured with the human P1-specific T cells with no human APCs added, there was little lymphoproliferation (SI = 0.8). That means human T cells cannot be activated by direct recognition of the xenogeneic antigens in this experimental system.

Tab 4. Lymphoproliferation of the Yn-P1-specific T cells to the xenogeneic SLA-expressed chondrocytes and PBMCs

combination*	exp 1		exp 2	
	cpm**	SI***	cpm	SI
hT	151±35	/	126±48	/
hT + hAPC	356±63	1.0	396±63	1.0
hT + hAPC + sChC	1130±133	3.2	1241±261	3.1
hT + hAPC + sPBMC	2088±265	5.8	/	
hT + sPBMC	221±54	0.6	/	

* hT: Yn-P1-specific human T cells; hAPC: irradiated human PBMCs;
   sPBMC: irradiated swine PBMCs; sChC: irradiated swine chondrocytes
** cpm: count per minute of ^{3}H-TdR uptake (mean±SD)
*** SI: stimulation index = cpm of exp combination / cpm of control (hT+hAPC)

DISCUSSION

The three kinds of Chinese pigs in our study are not inbred strains, but they are maintained in a highly isolated condition for a long time in the breeding stations. As indicated, for example, two of them are stable with an intra-strain similarity coefficient as high as 0.928 and 0.933[10]. It is thus not surprised that only one allele was detected in our study for each P1 and P14 locus of the three pigs. It is of course still possible that other alleles might be existed and could be detected.

To solve the problem that the damage of blood vessel endothelium may caused by the discrepancy between the human KIR and the swine class I molecule, Sasaki and his colleagues introduced HLA-G, a non-classical human MHC gene with little polymorphisms, into pig endothelial cells[18]. In this way, a human ligand suitable for all kinds of human KIRs was inducible to be expressed on the wall of pig blood vessel and the xeno-graft was free from NK's killing owing to the re-evoking a KIR-initiated inhibitory signal. Since there are only two amino acids substituted on the essential sequences of Chinese pigs' class I molecule for human KIR recognition in our study, whether the human KIRs are still able to deliver the inhibitory signal need a functional test.

T cells recognize allogeneic or xenogeneic antigens either by using direct or indirect pathway[7,19]. It was reported that both pathways could be adopted in recognition of SLA antigens by human T cells[7], but

only indirect pathway is adopted by human T cells for H-2 antigens[22], a fact corresponding to the differential distances detected in our study between species: human vs pig than human vs mouse (Tab 3). The main criterion to distinguish the two pathways is the origin of APCs involved. In case of direct recognition, donor's cells can work as both stimulators and APCs. For indirect recognition, however, only APCs from recipients are functioned. Tab 4 shows that in our study the SLA P1-specific T cells respond quite well to whole SLA class I antigens constitutively expressed on swine chondrocytes. This fact not only functionally confirms our engineering-expressed P1 antigen is able to work with satisfied immunogenicity but also gives a circumstantial evidence that the effect of the substitution of two crucial residues in swine class I molecule is strong enough to damage the collaboration between the human CD8 coreceptor and the swine class 1 antigens, which might force the CD8-positive T cells to use the indirect pathway to recognize the SLA antigens in same manner for recognition of conventional antigens.

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