Cloning and Characterization of a New Swine MHC (SLA) Class II DQB Allele

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ABSTRACT. Major histocompatibility complex (MHC) of pigs is known as swine leukocyte antigen (SLA). The cDNA encoding a new allele of SLA class II DQ ß-chain was successfully isolated from a CSK miniature pig (derived from Göttingen strain) and characterized by sequence analyses. SLA-DQB cDNA fragment encoding ß1-domain was amplified by reverse transcriptase-polymerase chain reaction using the sequences preserved in a various vertebrates as primers. Using non-radioisotope technique with the PCR product as a probe, cDNA clone G01 was isolated from a spleen cDNA library, and nucleotide sequence of this clone was determined. This clone encompassed a whole SLA-DQ ß-chain coding region, containing a total length of 1161 nucleotides with an open reading frame (ORF) of 786 nucleotides, 5‘ untranslated region of 15 nucleotides, and 3‘ untranslated region of 360 nucleotides ending with a canonical polyadenylation signal, followed by a poly A tail. Sequence comparisons of the ORF of this clone with those of known SLA-DQB genes confirmed that this clone is a new allele (SLA-DQB*G01). Phylogenetic analysis of the nucleotide sequences of swine, human, and murine MHC class II genes indicated that SLA-DQB was more similar to HLA-DQB1 than H-2Aß. Comparison of the nucleotide and deduced amino acid sequences among SLA-DQB alleles showed that the SLA-DQ ß-chain polymorphism was found almost in ß1-domain which contains the antigenic peptide binding sites. — KEY WORDS: major histocompatibility complex (MHC) class II, phylogenetic tree, swine leukocyte antigen (SLA), SLA-DQ.


Major histocompatibility complex (MHC) molecules are cell surface-expressed, highly polymorphic glycoproteins which present antigenic peptides to T cells and thus initiate specific immune responses [12, 21]. The molecules fall into two classes (I and II), which differ in their structure, tissue distribution, and function. In general, MHC class II molecules are heterodimers consisting of ß-chain and ß-chain, and present peptides derived from exogenously acquired proteins to CD4+ T cells. The peptides are bound to two membrane-distal domains (ß and ß) which are assumed to form a deep cleft made up of eight strands of antiparallel ß-sheet as a floor and two antiparallel ß-helices as the sides [1]. In humans, the class I molecules are encoded in A, B, and C regions, and the class II molecules are encoded in D region which contains three major regions, DP, DQ, and DR. In other mammals homologues to DP, DQ, and DR genes have been detected by genomic hybridization and/or molecular cloning. It has not yet been clarified whether the products of the individual MHC loci in different species perform identical functions during immune responses. Comparative structural studies of MHC genes between loci and/or between different mammalian species should assist in clarifying this issue.

The importance of immunological research of the pig has arisen because of its physiological relevance to human and its significance as a commercial animal. The miniature pig has been developed and shown to be an excellent experimental animal. Three inbred miniature pigs with different swine leukocyte antigen (SLA) haplotype, a, c, or d were developed at the National Institute of Health (NIH) in U. S. A. [16]. Most of the information on SLA have been obtained from researches of NIH miniature pigs. The SLA genes have been localized to chromosome 7 [5, 20], and genetic analysis indicates that the complex comprises class I, class II, and class III genes [11, 14, 25]. A closed colony of miniature swine/CSK (derived from Göttingen strain) has been established and maintained in Japan. In order to study more fully the SLA in evolutionary and functional respects, cloning and characterization of new alleles of SLA class II gene should be done. We carried out the isolation and genetic analyses of a new allele of SLA class II DQB.

MATERIALS AND METHODS

Animal: Miniature swine/CSK is derived from Göttingen strain and maintained as closed colony in CSK Research Park, Inc. (CSK Research Park, Inc., Nagano, Japan). Whole blood and spleen were obtained from these animals.

Amplification of SLA class II ß1-domain coding region: Total RNA was extracted from peripheral blood leukocytes (PBL) of a miniature pig by acid guanidinium thiocyanate-
were aligned, and genetic distances between them were determined using the nucleotide sequence of the product was identical to that of a full-length cDNA template. Two PCR primers, 5'-TGCTCAGTGACAGATTCTATCC-3' and 5'-AGGGCTGGG TGTGCTCACGTGACG-3', which were designed on the basis of DNA sequence of HLA-DQB1*0302, correspond to two conserved blocks of amino acids adjacent to the cysteine residues in the MHC class II β2-domain [9]. The conditions of amplification were 35 cycles of 1 min at 95˚C, 2 min at 55˚C and 2 min at 72˚C, and a final extension of 10 min at 72˚C. PCR product was cloned and used as a probe for screening of a cDNA library.

Construction and screening of a spleen cDNA library: A cDNA library was constructed from 5 μg of spleen poly(A)+ mRNA using a ZAP-cDNA SYNTHESIS KIT (Stratagene, San Diego, CA) and a GIGAPACK II PACKAGING EXTRACT (Stratagene). The spleen cDNA library was screened by plaque-hybridization. The probe was a cloned PCR product labeled with fluorescein-dUTP by enhanced chemiluminescence “Probe-amp reagent kit” (Amersham Corp., Arlington Heights, IL) according to the manufacturer’s manuscript. Screening was done by non-radioisotope (RI) plaque-hybridization method under stringent conditions, with the hybridization and washing at 65˚C.

Sequencing and sequence analyses: The recombinant plasmids were rescued from the positive phage clones, and the nucleotide sequence was determined by the dideoxy chain-termination method. Sequence data were processed by means of DNASIS™-Mac (HITACHI Co., Ltd., Kanagawa, Japan) on a Macintosh personal computer. Sequence similarities were assessed by the method of Lipman and Pearson [15] using GENETYX-Mac Homology program (Software Development Co., Ltd., Tokyo, Japan).

Phylogenetic analysis: Nucleotide sequences of MHC class II β-chains in swine, human (HLA), and mouse (H-2) were aligned, and genetic distances between them were calculated by the 6-parameter method of Gotoh et al. [6]. Genetic distances were determined using the nucleotide sequences in open reading frame (ORF) except for nucleotides which are consistent with the antigenic peptide binding sites (ABS) in HLA-DRB1*0101 [1]. Phylogenetic tree was constructed by neighbor-joining method [22] using the BIOPROSEARCH/SINCA computer program (Fujitsu Co., Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

To obtain cDNA templates for PCR amplification, total RNA of PBL was reverse-transcribed using the oligo d(T)16 primer. The cDNA thus synthesized was subjected to PCR with the primers described in “materials and methods”. After 35 cycles of amplification, only one major band of 191 base pairs (bp) was obtained. The purified PCR product was cloned into plasmid vector and sequenced. The nucleotide sequence of the product was identical to that of a part of SLA-DQBc–d. The product was, therefore, used as a probe for the screening of the miniature swine spleen cDNA library. By sequencing of clones obtained, the longest clone G01 was found to contain a 5' untranslated region of 15 nucleotides, an ORF of 786 nucleotides, and a 3' untranslated region of 360 nucleotides ending with a polyadenylation signal, followed by a poly A tail, with a total length of 1161 nucleotides. The ORF started with the initiation codon ATG and was terminated by the stop codon TGA (Fig. 1).

So far, the only two full nucleotide sequences of ORF of SLA-DQB (SLA-DQBc and SLA-DQBd) of NIH miniature pig, in addition to five partial sequences of SLA-DQB of different alleles (SLA-DQB*S01–S05), have been reported [8, 23]. Comparison of G01 with known SLA-DQB alleles confirmed the assignment of G01 as a new SLA-DQB allele (SLA-DQBc*G01) (Figs. 1 and 2).

To characterize the primary structure of SLA-DQ β-chain, the full sequence of nucleotides and deduced amino acids of three SLA-DQB alleles were aligned in Fig. 1. The nucleotide and deduced amino acid sequences of the ORF of SLA-DQBc*G01 were highly similar to those of SLA-DQBc (97.0% and 93.0%, respectively) and SLA-DQBd (97.5% and 94.8%, respectively). Sequence similarities in SLA-DQ β2-domain were 93.3–94.0% at the nucleotide level and 85.1–88.3% at the protein level, whereas those in the remainder of the ORF were 99.5–99.8% at the nucleotide level and 98.5–99.3% at the protein level. That is, in SLA-DQ β-chains, the amino acid differences were almost found in the β2-domain including ABS as similar as HLA-DR reported by Brown et al. [1]. In the β1-domain coding region, there were 17 nonsynonymous (amino acid-altering) substitutions and only 2 synonymous substitutions between SLA-DQBc*G01 and SLA-DQBd, and 14 nonsynonymous substitutions and 3 synonymous substitutions between SLA-DQBc*G01 and SLA-DQBd. It is suggested, therefore, that maintenance of polymorphism in the SLA class II β2-domains is influenced by positive selection, like in human and murine MHC class II molecules [10, 19].

Phylogenetic tree of nucleotide sequences of MHC class II β-chain in swine, human, and mouse was constructed with the neighbor-joining method (Fig. 3). The phylogenetic tree showed that the SLA class II genes were evolutionary closer to HLA class II genes than H-2 genes. Phylogenetic analysis of MHC genes might provide an understanding of how the immune system evolved. This analysis, therefore, indicated that divergent evolution in mammalian species was after the divergence of MHC class II DQ and DR.

For comparative analysis among swine, human, and mouse MHC class II DQs, alignment of amino acid sequences of DQ β-chain of each species was shown in Fig. 2. In β2-domain, the number of the amino acid residues shared by SLA-DQ β and HLA-DQB1 (8 residues) was more than that of the residues shared by SLA-DQ β and I-A β (2 residues) or HLA-DQB1 and I-A β (4 residues). In constant region, including β2-domain, connecting peptide, transmembrane region, and cytoplasmic region, SLA-DQ β shared 15 residues with HLA-DQB1 and only 4 residues...
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with I-Aβ, on the other hand the residues shared by HLA-DQB1 and I-Aβ were 13. Furthermore, both SLA-DQβ and HLA-DQB1 lack of the residues at the same position in cytoplasmic region, which are at the codon position 226–233 in the murine I-Aβ. The swine immune system controlled by SLA class II molecules, therefore, might be more similar to that of human than that of mouse.

The information on SLA-DQβ-chain in this study could greatly contribute to study of SLA class II molecules which is behind those of human HLA and murine H-2. Using SLA-DQB cDNA clone, detection of expression at the mRNA level and analysis of function of gene product will be carried out. For the investigation of the function of SLA class II molecules in immune system of miniature pig, more information of these molecules is necessary. Further characterization of SLA class II molecules, including not only DQ but also DR and/or DP molecules, is now ongoing.

The nucleotide sequence data reported in this paper has been submitted in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB010577.

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Fig. 1. Comparison of SLA-DQB*G01 with SLA-DQB8 and -DQB8 sequences [8] in the ORF. Both nucleotide and predicted amino acid differences among the three sequences are shown. Dashes indicate identities as compared to SLA-DQB*G01 sequence. Solid triangles indicate cysteine residues involved in disulfide bridges, and a putative N-linked glycosylation site is underlined. Plus symbols denote the amino acid positions constituting part of the ABS of HLA-DRB1 described by Brown et al. [1].
Fig. 3. Phylogenetic tree of nucleotide sequences of MHC class II β-chains in swine, human, and mouse constructed by neighbor-joining method [23]. Sequences used in the calculation of genetic distances were 714 bp long in ORF except for ABS coding region. A bar represents the branch length which is proportional to the genetic distance. Sequences used in this figure are quoted from the following references: [7, 23] for SLA-DQ β, [18] for HLA-DQB1, and [3, 13, 17] for I-A β.

Fig. 2. Comparison of MHC class II DQ β-chains in pig (SLA-DQ β), human (HLA-DQB1), and mouse (I-A β) in β1-domain, β2-domain, connecting peptide (CP), transmembrane region (TM), and cytoplasmic region (CY). □: residues shared by SLA-DQ β and HLA-DQB1, □: residues shared by SLA-DQ β and I-A β, and □□: residues shared by HLA-DQB1 and I-A β. Plus symbols denote the amino acid positions constituting part of the ABS of HLA-DRB1 [1]. Amino acid sequences used in this figure are quoted from the following references: [8, 23] for SLA-DQ β, [18] for HLA-DQB1, and [3, 13, 17] for I-A β.


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