Analysis and characterization of the swine major histocompatibility complex

Wen-Rong Lie
Iowa State University

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Analysis and characterization of the swine major histocompatibility complex (SLA)

Lie, Wen-Rong, Ph.D.
Iowa State University, 1987
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the swine major histocompatibility complex

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Wen-Rong Lie

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GENERAL INTRODUCTION

Major Histocompatibility Complex in Mouse and Human (H-2 and HLA)

The histocompatibility antigens were originally discovered in the mouse nearly 50 years ago (1,2) as the cell surface proteins that trigger allograft rejection. These genetically determined antigens have been found to be encoded by a group of closely linked genes, called the major histocompatibility complex (MHC) (reviewed in 3). Since its discovery, many investigators have elucidated a great deal of the MHC's genetics, structure and function. The importance of the role of the MHC in modern science was acknowledged when the 1980 Nobel Prize in Physiology and Medicine was awarded to Baruj Benacerraf, Jean Dausset and George Snell for their work on the MHC (4).

An MHC has been described in most mammals studied (5-7). A high degree of homology of the MHC among various species has been found. The exact number of genes within the MHC and its true boundaries are not known. At least three sets of molecules, designated class I, class II, and class III, have been described based on the structural and/or functional characteristics of the gene products (8,9). The class I and class II molecules are cell surface proteins which regulate the immune response, whereas the class III molecules include
serum complement components. Genetic polymorphisms of MHC molecules have been linked to the susceptibility of an individual to certain diseases (10-15), however, the mechanism underlying this association has not been fully established (16,17). Recently, the rapid progress made in hybridoma technology and recombinant DNA techniques has led to a better understanding of the biochemistry of MHC products, the molecular biology of MHC genes, and the role of the MHC in immunological self/nonself discrimination. The best studied mammalian MHCs are those of the mouse (H-2) and man (HLA) (18-28). The H-2 complex is found on chromosome 17 in the mouse (Figure 1) and the HLA complex is on chromosome 6 in the human (Figure 2).

**MHC class I molecules**

MHC class I molecules are membrane bound glycoproteins which are found on most cells of the body. The characteristic feature of these molecules is a highly polymorphic 45 kd chain noncovalently associated with a 12 kd chain called \( \beta_2 \) microglobulin (\( \beta_2m \)) (Figure 3A). \( \beta_2m \) is encoded by a non-MHC gene. In the mouse, three distinct MHC class I loci have been identified, designated H-2K, H-2D, and H-2L. The comparable human loci are HLA-A, HLA-B, and HLA-C. These class I molecules are the transplantation antigens and act as restriction elements for antigen recognition by cytotoxic T cells (29). In the mouse there is another category of class I
Figure 1. A molecular map of the MHC of the BALB/c mouse (H-2 haplotype d). An estimated 2300 kb of DNA are correlated with the genetic regions and subregions defined by recombination events. H-2 genes and classes are indicated. The distance between the class II and the class III genes, not yet cloned, is approximately 170 kb and the distance between the class III genes and the TNF-α and -β (tumor necrosis factor) genes is at least 350 kb. Centimorgan (cM) distances are indicated, 21-OH denotes 21-hydroxylase [modified from ref. (23, 24)]
Figure 2. A genetic map of the human HLA complex. Genetic regions, classes, HLA genes and map distances in cM (centimorgans) are indicated. The HLA-A, B, and C loci determine the class I molecules. The HLA-DR, DQ, and DP loci determine class II molecules. The order of the genes in the class II subregions is uncertain. Each class II subregion (DR, DQ, DP) contains at least one functional α/β heterodimer. 21-OH indicates 21-hydroxylase. TNF denotes tumor necrosis factor and its position has been tentatively assigned between the class I and class III loci. The orientation of the class III genes with respect to the class I and class II genes is not known [modified from ref. (25-28)]
Figure 3. Schematic representation of (A) a class I molecule and (B) a class II molecule. The class I molecule consists of a heavy chain (MW ~45 kd) associated non-covalently with β₂m (β₂ microglobulin, MW ~12 kd). The three external domains of the heavy chain, termed α₁, α₂, and α₃, are shown. β₂m is coded for by a non-MHC gene. The class II molecule consists of an α chain (MW ~33 kd) non-covalently associated with a β chain (MW ~28 kd). Each class II chain has two external domains denoted α₁ and α₂ or β₁ and β₂. CHO is carbohydrate. S-S indicates the presence of disulfide bridges [modified from ref. (19)]
(A) Class I

(B) Class II
molecules, designated Qa and Tla antigens, which are less polymorphic and are uniquely expressed on specific cell types (9). Molecular genetic data reveal that most class I genes map to the Qa and Tla regions and comparatively few to the H-2K and H-2D regions. The functions of Qa/Tla antigens are unknown.

Molecular cloning of the class I cDNA or genomic DNA have answered some of the fundamental questions concerning the number, organization, and expression of these class I genes. A typical class I gene is composed of seven or eight exons of which three encode the three external domains, α₁, α₂, and α₃ (Figure 4A). The nucleotide sequence data for some class I molecules have also been established. The reexpression of cloned genes in the selected host cells has raised the possibility for a new level of functional study of individual MHC products.

MHC class II molecules

The class II molecules have a much more limited distribution than class I molecules. They are found only on certain immune cells, including B cells, macrophages, and some T cells. Class II molecules are membrane bound glycoproteins composed of two polypeptide chains, an α chain of approximately 33 kd and a β chain of 28 kd (Figure 3B). Both chains are encoded by genes within the MHC complex. The
Figure 4. Schematic representation of the exon-intron organization of (A) class I and \( \beta_2m \) (\( \beta_2 \) microglobulin) genes and (B) class II \( \alpha \) and \( \beta \) genes. Black boxes represent exons for coding sequences and hatched boxes are exons for 3' untranslated regions. Interconnecting lines indicate introns. L, TM, CP, and 3'UT denote leader sequence, transmembrane sequence, cytoplasmic sequence and 3'untranslated region respectively [modified from ref. (19)]
(A) Class I Gene

(B) Class II \( \alpha \) Gene

(B) Class II \( \beta \) Gene
difference in molecular weight is mainly attributed to glycosylation. In the mouse, two distinct types of class II molecules, I-A and I-E, have been described. The $\alpha_\alpha$, $\alpha_\beta$, and $\beta_\beta$ molecules are highly polymorphic, whereas the $\beta_\alpha$ molecule is not. In humans, three types of class II molecules, designated DR, DQ, and DP have been identified. The function of these class II molecules is to present processed foreign antigen to helper T cells and suppressor T cells (30).

The cloning and sequence analysis of the class II genes has also been performed. A correlation between the organization of exons and structural domains of the class II molecules has been observed (Figure 4B). The "rules" for $\alpha\beta$ chain pairing are not completely known at present.

**MHC class III molecules**

The MHC class III molecules include three complement components, C2, factor B (Bf), and C4; which are secreted primarily in the liver as soluble molecules and circulate in the plasma (reviewed in 28). C4 is a single glycoprotein chain of approximately 200 kd (Figure 5) which is processed before secretion to give three chains ($\alpha$ 95 kd, $\beta$ 75 kd, $\gamma$ 30 kd). C2 and Bf are also glycoproteins which are synthesized and secreted as single peptide chains of about 90-100 kd (Figure 5). The observed polymorphisms of C4 are greater than those for the C2 and Bf but less than those of the class I and class II molecules. These class III molecules are involved
Figure 5. Schematic diagram illustrating the protein structure of the C4, C2, and Bf molecules [modified from ref. (28)]
(A) C4

\[ \alpha \text{ NH}_2 \quad C4a \quad C4d \quad \text{COOH} \]

\[ \beta \text{ NH}_2 \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{COOH} \]

\[ \gamma \text{ NH}_2 \quad \text{S} \quad \text{COOH} \]

(B) C2

\[ \text{NH}_2 \quad C2b \quad C2a \quad \text{COOH} \]

(C) Factor B (Bf)

\[ \text{NH}_2 \quad \text{Ba} \quad \text{Bb} \quad \text{COOH} \]
primarily in humoral immunity. The C2 and C4 components are important in the classical complement activation pathway and Bf is important in the alternative pathway (Figure 6) (31). The Slp in the mouse is a hemolytically inactive homolog of C4 with unknown function (32).

Most of the human and mouse class III genes have been cloned and sequenced (33,34). The C2 and Bf genes are very close in proximity in the class III region. There are two C4 genes in the class III region which are C4A and C4B in humans and are C4 and Slp in mice. Recently, duplicated genes of 21-hydroxylase (21-OHase), which encode an enzyme used in the steroid synthetic pathway, have been found 3' of the two C4 genes in both mice and man (35-37).

Other MHC linked molecules

Some enzyme-encoding loci have been linked to the MHC (reviewed in 3). In the mouse, they are Ce-2 (kidney catalase-2), Cld (combined lipase deficiency), Acry-1 (a-crystallin), Glo-1 (glyoxalase-1), Mep-1 (meprin), Neu-1 (neuraminidase-1), Pgk-2 (phosphoglycerate kinase-2), Sod-2 (superoxide dismutase-2), and Upg-1 (urinary pepsinogen-1). In man, HLA linked enzyme-encoding loci are factor XIII, Glo1 (glyoxalase 1), Mel (malic enzyme), PGM3 (phosphoglucomutase). Among these enzyme loci, glyoxalase-1 is associated with MHC
Figure 6. Schematic diagram illustrating two pathways of activation of complement. The classical pathway is activated primarily by antibody-antigen aggregates when C1 binds to the Fc portion of the aggregated antibody. This leads to the activation of C4 and C2 to form C4b and C2a. C4b binds covalently with antibody and antigen and C2a associates with it non-covalently. C4b2a acts as a C3 convertase to form C3b. The C4b2a3b complex catalyses activation of C5 to form C5b. C5b initiates formation of the complex C5b6789 which can lyse adjacent cells. The alternative pathway is activated by antibody-antigen complexes as well as other substances such as high molecular mass polysaccharides found in bacterial and yeast cell wall. Activated C3 binds covalently to aggregates or polysaccharides. When factor B (Bf) associates with it, in the presence of factor D (D) and properdin (P), it forms C3bBb which is a C3 convertase and activates C3. Binding of another C3b molecule forms C5 convertase, C3bBb3b, which initiates formation of the same membrane attack complex as in the classical pathway [modified from ref. (28)]
CLASSICAL ALTERNATIVE

C1 $\rightarrow$ C1$\tilde{\varepsilon}$

C3 $\rightarrow$ C3b $\rightarrow$ C4b2a $\rightarrow$ C3a $\rightarrow$ C3b $\rightarrow$ +Bf $\rightarrow$ D, P

C3b $\rightarrow$ C3b +Bb $\rightarrow$ C4b2a $\rightarrow$ C3b 3b

C5 $\rightarrow$ C5b $\rightarrow$ C5a

C6, C7 $\rightarrow$ C5b 67

C8, C9 $\rightarrow$ C5b 6789 (lysis)
in both mice and humans. It is not known if the linkage of
these enzyme encoding loci with the MHC is significant.
However, these loci have served as useful markers in
recombinational analysis and in population studies. There are
some other loci which have been reported to be linked to the
MHC, e.g., C3 and T/t complex in mice (38-39) and PRL
(prolactin) in humans.

The recent gene map of the H-2 and HLA complexes, which
has been created by molecular cloning and chromosomal walking,
has also placed 21-OHase genes, the tumor necrosis factor
(TNF-α) gene and the lymphotoxin (TNF-β) genes within the MHC
complex (27,35-37,40). It has been postulated that more
genomes, still to be cloned, will be found within the MHC.

Major Histocompatibility Complex in Swine (SLA)

The swine MHC, the SLA complex, was first described by
Vaiman et al. and Viza et al. in 1970 (41,42). Interest in
swine as a model for transplantation experiments stimulated
the original studies on SLA (43-45). Since then, the SLA
complex has been studied in both commercial swine breeds and
lines of miniature swine (reviewed in 46).

Analyses of the structure of the class I and class II
molecules and the genes of the SLA complex have been initiated
in several laboratories, including ours. The SLA complex has
been reported to be on swine chromosome seven by in situ
hybridization (47,48). Similarities have been found between
the SLA complex and the MHC of various other mammalian species (Figure 7).

The economic value of swine has led to a search for associations between SLA and disease susceptibilities (49-52) and between SLA and production traits (53,54). The early work on SLA typing was performed with the use of alloantisera or mixed lymphocyte reactions (55-58). Recently, production of monoclonal antibodies (MAbs) to recognize SLA class I and class II antigens has been initiated (59-61). Restriction fragment length polymorphism (RFLP) analysis of SLA genes for SLA typing at the DNA level, has also been performed in both miniature and commercial swine by cross hybridization of swine genomic DNA with human and/or mouse MHC DNA probes (45,62-64).

SLA inbred miniature swine

Miniature swine have been used as animal models in biomedical research because of their small adult size (150-200 pounds), and similarity to human physiological systems. A herd of SLA inbred miniature swine, originated by Sachs et al. at the NIH Transplantation Biology Unit, has been developed and is an excellent model for studying the role of SLA in allograft survival (45). The selection of these NIH miniature swine has been based on class I antigen types, MLR studies and results of immunization among siblings. The first two miniature swine in the selective breeding were given the
Figure 7. Schematic diagram of the SLA complex. The relative position of different genes within each class of SLA is unknown; positions shown in the diagram are based on those reported for HLA complex. The exact location of C4 in the SLA complex is uncertain [modified from ref. (46)]
arbitrary SLA haplotypes of \( ab \) and \( cd \). Only \( ac \) and \( ad \) progeny were found in the first litter. Selective breeding of several generations has led to the successful production of three SLA-homozygous lines which express the three independent SLA haplotypes, \( a, c, \) and \( d \). Two independent intra-SLA recombinants, \( f \) and \( g \) have also been derived from the three original haplotypes (65). Both recombinants express the class I genes of the \( c \) haplotype and the class II genes of the \( d \) haplotype. The schematic diagrams of these SLA haplotypes are shown in Figure 8.

NIH miniature swine have provided an excellent model for transplantation research. Allograft survival is increased by matching graft and host SLA types (66,67). The SLA inbred and recombinant swine have also been shown to be very useful in the analyses of the structure and function of the SLA complex (46).

**SLA class I molecule**

The structure and cell distribution of SLA class I antigens are similar to those reported in humans and mice. Biochemical analysis has shown that the SLA class I antigen consists of a polymorphic glycoprotein of approximately 44 kd noncovalently associated with the non-MHC encoded 12 kd \( \beta_2m \) (68-70). Three class I loci have been identified and designated as SLA-A, SLA-B, and SLA-C (71-73). Haplotype associated restriction fragments have been detected
Figure 8. Schematic representation of SLA haplotypes of inbred and recombinant miniature swine. The specific allele inherited in each haplotype is denoted by the appropriate box. The SLA a, c, and d are three independent SLA haplotypes. The ÷ and £ are recombinants derived independently. It is not known where the recombinant point is within ÷ or £ haplotypes [modified from ref (46)]
SLA Haplotypes

\[ \text{SLA}^a = a = \]
\[ \text{SLA}^c = c = \]
\[ \text{SLA}^d = d = \]

SLA Recombinants

\[ \text{SLA}^f = f = \]
\[ \text{SLA}^g = g = \]
by the RFLP analysis of class I genes of domestic and miniature swine (46,62). No class I MHC restriction of antigen recognition has been reported in swine.

Porcine class I genes, from miniature swine of the d haplotype, have been isolated and characterized (74). At least three of the class I genes, PD1, PD14, and PD6 have shown expression in mouse L cells after transfection (75). The PD1 gene has also been microinjected into mouse embryos to produce transgenic mice (76). DNA sequence data have shown that the SLA class I genes contain the typical class I intron/exon structure.

**SLA class II molecules**

SLA class II antigens are expressed on macrophages, B cells and some T cells (77). The class II molecules are composed of two glycoproteins, an α chain of approximately 32 kd and a β chain of approximately 25 kd (78,79). Two class II products, SLA-DQ and SLA-DR, have been identified. Associations of the SLA class II region and immune responses to (T,G)-A-L and to lysozyme have been well established in SLA inbred and recombinant miniature swine (46,80).

No porcine class II genes have been isolated. RFLP analysis of genomic DNA from domestic and miniature swine has been performed using human class II α and β specific probes (46,62). DNA hybridization data suggest that the existence of other class II genes in swine is possible.
SLA class III molecules

Less is known about class III genes in swine than in mice and humans. Total hemolytic complement levels have been associated with certain SLA haplotypes (81). Recently, C4 has been mapped to the SLA complex by detecting C4 RFLPs in domestic swine (82). The exact location of C4 in the SLA complex is uncertain. No studies published have described the mapping of swine C2 and Bf.

Other SLA linked loci

Association of the SLA complex with other markers is not well established. It has been shown that the polymorphic enzyme locus PGM3 may be linked to the SLA complex (83). Association of SLA and blood group loci C, J, and H has also been reported (84,85). Because of the preestablished linkage between the loci for glucosephosphate isomerase (GPI), halothane sensitivity (HAL), A-O inhibition (S), H blood groups, postalbumin-2 (PO2) and 6-phosphogluconate dehydrogenase (6PGD), it has been postulated that the loci for SLA, C, J, GPI, HAL, S, H, PO2 and 6PGD may form one large linkage group (86). However, the recent mapping of the loci of H blood groups to chromosome 15 (87), not chromosome 7, has shown that some questions on the possible existence of SLA-C-J-GPI-HAL-S-H-PO2-6PGD linkage does not exist.
Research Goals

The research goals for this dissertation were:

1. Detection of a SLA linked enzyme marker: Study the possible linkage between the Glo enzyme locus and the SLA complex.


SECTION I. QUANTITATIVE DIFFERENCES IN GLO ENZYME LEVELS ASSOCIATED WITH THE MHC OF MINIATURE SWINE
QUANTITATIVE DIFFERENCES IN GLO ENZYME LEVELS ASSOCIATED WITH THE MHC OF MINIATURE SWINE

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INTRODUCTION

The enzyme glyoxalase-I (GLO) (EC4.4.1.5) catalyses the conversion of methylglyoxal and reduced glutathione to S-lactoylglutatione (1). The linkage between Glo and the major histocompatibility complex (MHC) has been demonstrated in man (2) and in the mouse (3), based on the analysis of electrophoretic polymorphic phenotypes of GLO. This conserved linkage also has been reported in rats, which have no GLO electrophoretic polymorphisms but do have quantitative differences in GLO enzyme activity (4).

In domestic animals, a search for GLO polymorphisms in swine and cattle has failed to detect any electrophoretic variants (5). In the present study, we report the search for electrophoretic variants and quantitative enzyme activity level differences in miniature swine of known SLA haplotypes.
MATERIALS AND METHODS

Animals and Experiments

Miniature swine, representing three independent SLA haplotypes, SLA^a/a, SLA^c/c and SLA^d/d and an intra-MHC recombinant haplotype g/g were introduced into our herd from the NIH Animal Center (6, 7). These animals were bred and maintained at the Iowa State University Bilsland Swine Breeding Farm. Twenty-five miniature swine, which included SLA^a/a, SLA^c/c, SLA^d/d and SLA^g/g homozygous haplotypes and some heterozygous haplotypes were used for the detection of electrophoretic GLO polymorphisms. A total of 18 outbred pigs from five breeds of US pigs (Chester White, Duroc, Hampshire, Landrace and Yorkshire) were also tested for electrophoretic GLO variants.

In the first experiment, the amount of GLO activity in red blood cells of miniature swine of SLA^a/a, SLA^c/c and SLA^d/d haplotypes (eight pigs total) was compared. A second experiment to further test the relationship between GLO activity and haplotypes SLA^a/a, SLA^a/d and SLA^d/d was conducted using seven sets of littermates from three SLA^a/d F_1 crosses and four backcrosses (29 pigs total). A two-stage antibody and complement-dependent microcytotoxicity test was used routinely for SLA typing (8).
Electrophoresis

The search for GLO polymorphisms was accomplished by electrophoresis on cellulose acetate gels. Blood (2 ml) was collected from the retro-orbital plexus of minipigs into heparinized tubes. Erythrocytes were obtained by removing the buffy coats and washing three times with 0.15 M saline. These washed pellets were haemolysed with an equal volume of distilled water. Clarified haemolysates (0.5 µl) were then applied onto cellogel 250 strips (Kalex). The electrophoresis was run at 5 mA/strip for 30 min at 4°C using 0.015 M phosphate buffer, pH 6.8 (9). After being run, gel strips were incubated with staining agar [18.2 mM reduced glutathione (Sigma), 670 mM methylglyoxal (Sigma) in 0.2 M potassium phosphate buffer containing 21.2 mM MgCl$_2$, mixed with an equal volume of 1.6% molten agarose containing 3.8 mM MTT] at 37°C for 60 min. A colorless band appeared at the site of the GLO reaction.

Enzyme Activity Assay

A quantitative assay of GLO activity was done by minor modifications of the procedures of Rajan (10), Stolc et al. (4), and Rubinstein and Vienne (11). A brief description is included. Washed red cell pellets were lysed on ice by addition of 9 volumes of 5 mM HEPES buffer, pH 7.0. A further 1:50 dilution of the clarified lysates was made in 25 mM HEPES buffer, pH 7.0, before each assay. Total protein and
haemoglobin (Hb) concentrations of these lysates were
determined by the Lowry procedure (12) and cyanmethaemoglobin
method (13), respectively. GLO activity was determined by
measuring the change in absorbance of the assay mix at 240 nm
(10 min, 28°C) due to the appearance of S-lactoylglutathione
(E = 3370 mol\(^{-1}\) cm\(^{-1}\)). A dual-beam Cary 118 recording
spectrophotometer was used in which the reference cuvette and
the enzyme-containing cuvette were water-jacketed. In each
assay, 50 \(\mu l\) of diluted haemolysate were added into a cuvette
containing 1.15 ml of substrate solution (50 \(\mu l\) of 58 mM
methylglyoxal in distilled water and 1.1 ml of 2.3 mM reduced
glutathione in 0.05 M phosphate buffer, pH 6.8). The blank
cuvette contained 1.15 ml of substrate and 50 \(\mu l\) of 25 mM
HEPES buffer. The change in absorbance was linear for at
least 15 min. These data were computed and expressed in terms
of units/mg protein and units/mg Hb (1 unit = the amount of
enzyme activity generating 1 \(\mu\)mol of S-lactoylglutathione per
min). In this assay, animals in each litter were analyzed on
the same day. Two pigs, one SLA\(^a/a\) and one SLA\(^d/d\), were run
as controls on each experimental day. Two blood samples were
collected from each pig and the GLO activity was analyzed
separately for each sample. Results were then averaged. The
identity of the SLA haplotype of each pig was unknown at the
time of the GLO enzyme assays. Data were analyzed by using
least-squares analysis of variance with a model that included
the effects of litters, haplotypes and the interaction of litters and haplotypes. Estimated means for each haplotype were computed. Comparison of means was made by using Fisher's LSD after a significant F test was found for haplotype differences.
RESULTS AND DISCUSSION

The results of electrophoresis demonstrated a single band of porcine GLO activity, which migrated anodally at a rate greater than both GLO\textsuperscript{a} and GLO\textsuperscript{b} variants in mice (Fig. 1). We also tested several outbred pigs from each of five breeds of pigs (Chester White, Duroc, Hampshire, Landrace and Yorkshire) and found no evidence of GLO polymorphisms (data not shown). This lack of GLO electrophoretic phenotypes in outbred pigs agrees with the results from Vogel et al. (5).

Two alleles, Glo\textsuperscript{h} and Glo\textsuperscript{l}, which control high and low levels of enzyme activity in rats have been mapped to the rat MHC (RT1) at a distance of 4.6 cm from RT1.A by Stolc et al. (4). The discovery of this linkage in rats, by quantitative assays instead of electrophoresis, provided a new approach for investigating this genetic region in swine.

Initially, GLO enzyme activity in the red cell lysates from miniature swine of SLA\textsuperscript{a/a}, SLA\textsuperscript{c/c} and SLA\textsuperscript{d/d} were compared. The results are shown in Table 1. Eight minipigs were selected and grouped into three sets, each set including minipigs of approximately the same age but different haplotypes. Age was kept as constant as possible in each group to eliminate variability due to the variation of Hb concentration with age (14). There were no differences in GLO activity between male and female pigs of the same age. In all three groups, the comparison showed a consistently higher enzyme
Figure 1. Electrophoretic phenotypes of glyoxalase-I (GLO) in mice and miniature swine. 1. Mouse, C57BL/6, H-2^b. 2. Mouse, MA/MyJ, H-2^k. 3. Miniature swine, SLA^a/a. 4. Miniature swine, SLA^c/c. 5. Miniature swine, SLA^d/d. 6. Miniature swine, SLA^g/g. 7. Miniature swine, SLA^a/c. 8. Miniature swine, SLA^g/d.
Table 1. Glyoxalase-I (GLO) activity in SLA<sup>a/a</sup>, SLA<sup>c/c</sup> and SLA<sup>d/d</sup> in miniature swine

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Number Tested</th>
<th>Relative GLO activity per mg hemoglobin</th>
<th>Relative GLO activity per mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA&lt;sup&gt;a/a&lt;/sup&gt;</td>
<td>3</td>
<td>1.26</td>
<td>1.24</td>
</tr>
<tr>
<td>SLA&lt;sup&gt;c/c&lt;/sup&gt;</td>
<td>3</td>
<td>1.23</td>
<td>1.19</td>
</tr>
<tr>
<td>SLA&lt;sup&gt;d/d&lt;/sup&gt;</td>
<td>2</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

activity in minipigs of SLA<sup>a/a</sup> and SLA<sup>c/c</sup> haplotypes as opposed to the SLA<sup>d/d</sup> haplotype. Based on these results, and the availability of the required crosses, further experiments were conducted on pigs of the SLA<sup>a/a</sup> and SLA<sup>d/d</sup> haplotypes.

The second experiment, to further investigate quantitative differences in GLO activity, was conducted with the analysis of data from a total of 29 miniature swine, produced as shown in Table 2. The data from these seven sets of littermates, resulting from the F<sub>1</sub> and backcross matings (3 SLA<sup>a/d</sup> X SLA<sup>a/d</sup>, 2 SLA<sup>a/d</sup> X SLA<sup>a/a</sup> and 2 SLA<sup>a/d</sup> X SLA<sup>d/d</sup>), were analyzed by analysis of variance. The least-squares means of GLO activity of the different haplotypes are in Table 3. The association of higher activity with the a haplotype and lower activity with the d haplotype was found in each litter.

The ratio of GLO activity from SLA<sup>a/a</sup> minipigs to SLA<sup>d/d</sup> minipigs was consistently 1.3 with use of either total protein
Table 2. Experimental matings and progeny produced for the quantitative GLO activity analysis

<table>
<thead>
<tr>
<th>Number of matings</th>
<th>Mating type</th>
<th>Number of progeny produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SLA(^a/d) X SLA(^a/a)</td>
<td>SLA(^a/a)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

\(^a\)Experiment 2.

Table 3. Mean glyoxalase-I (GLO) activity in SLA\(^a/a\), SLA\(^a/d\) and SLA\(^d/d\) miniature swine

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Number tested</th>
<th>GLO activity units(^1)/mg haemoglobin (mean ± S.E.)(^2)</th>
<th>GLO activity units(^1)/mg protein (mean ± S.E.)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLA(^a/a)</td>
<td>8</td>
<td>0.216(^a) ± 0.004</td>
<td>0.167(^a) ± 0.003</td>
</tr>
<tr>
<td>SLA(^a/d)</td>
<td>14</td>
<td>0.188(^b) ± 0.003</td>
<td>0.148(^b) ± 0.002</td>
</tr>
<tr>
<td>SLA(^d/d)</td>
<td>7</td>
<td>0.164(^c) ± 0.004</td>
<td>0.130(^c) ± 0.003</td>
</tr>
</tbody>
</table>

\(^1\)One unit is the amount of enzyme activity generating 1 µmol of S-lactoylglutathione per min.

\(^2\)Least-squares mean ± standard error.

\(^a\),\(^b\),\(^c\)Means in the same column with different superscripts are statistically different (p < 0.01).
concentration or Hb concentration to standardize this assay.
This can be compared with a ratio of approximately 6.0 between
\( \text{Glo}^h \) strains and \( \text{Glo}^1 \) strains in rats (4). In mice, a ratio
of 5.1 was found between the activities of the highest
(\( \text{QGlo-1}^c \)) and the lowest (\( \text{QGlo-1}^a \)) strains (11). Table 3 also
shows that the \( \text{SLA}^{a/d} \) heterozygotes had an enzyme activity
intermediate to the \( \text{SLA}^{a/a} \) and \( \text{SLA}^{d/d} \) homozygotes. This
suggests that the differences in enzyme activity between
\( \text{SLA}^{a/a} \) and \( \text{SLA}^{d/d} \) are simply inherited. Analysis of within
litter data showed that GLO activity of individual pigs of
different haplotypes overlapped only twice in this data set
but average values for each haplotype within a litter never
overlapped. We conclude that a linkage of \( \text{Glo} \) to the \( \text{SLA} \)
complex is suggested on the basis of quantitative differences
in GLO levels among haplotypes. Since the ratio of \( \text{SLA}^{a/a} \) to
\( \text{SLA}^{d/d} \) is only 1.3 the usefulness of this linkage as a genetic
marker for the \( \text{SLA} \) complex may be limited.


SECTION II. MOLECULAR ANALYSIS OF SWINE CLASS III GENES.

I. MAPPING OF C2 AND BF GENES TO THE SWINE MAJOR HISTOCOMPATIBILITY COMPLEX (SLA)
MOLECULAR ANALYSIS OF SWINE CLASS III GENES.

I. MAPPING OF C2 AND BF GENES TO THE SWINE MAJOR
HISTOCOMPATIBILITY COMPLEX (SLA)

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Abbreviations used in this paper: RFLP, restriction fragment
length polymorphism; C2, C3, C4, second, third, fourth
components of complement; Bf, complement factor B; SLA, swine
leukocyte antigen; MHC, major histocompatibility complex.

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Newton, Young Chul Jung, Nancy Schwartz, and Janice Voetburg
for blood sample collection. We thank Ina Pour-El for the
technical assistance in the electroelution. We also thank Dr.
David Bentley for the pC201 clone, and Dr. Duncan Campbell for
the pFB3b clone.
ABSTRACT

Three miniature swine lines, inbred for SLA haplotypes \(a\), \(b\), and \(d\), and a recombinant line, haplotype \(g\), were analyzed for possible restriction fragment length polymorphisms (RFLPs) by Southern blot hybridization with human C2 and factor B (Bf) specific probes. The search for RFLPs by using a human C2 probe failed to reveal any variants. However, a TaqI polymorphism was identified with a human Bf probe. Overlapping restriction fragments were found with C2 and Bf probes, which strongly suggests close linkage of C2 and Bf genes in swine. Two TaqI RFLP patterns were distinguished with the Bf probe. Segregation analyses indicated that the polymorphic fragments followed a Mendelian pattern of inheritance. The recombinant haplotype \(g\), which expresses class I genes of haplotype \(c\) and class II genes of haplotype \(d\), was shown to produce an identical RFLP pattern, using the Bf probe, as haplotype \(d\), but different from that of haplotype \(c\). This indicates that there is a close association between Bf and class II genes and that the recombination event leading to the \(g\) haplotype must have occurred between the class II-Bf and class I genes. Although these data do not show conclusively the location of the C2-Bf genes, it is hypothesized that swine C2-Bf is located between the class II and class I genes, as has been demonstrated in mouse and man.
INTRODUCTION

The major histocompatibility gene complex (MHC) is a group of closely linked loci coding for highly polymorphic molecules that play a key role in the control of the functions of the immune system. Two major classes of MHC gene products, class I transplantation antigens and class II Ia antigens, have been described in all mammals investigated to date (1). In addition to the cell surface class I and class II antigens, a third group of polymorphic proteins, designated class III proteins, has been mapped between the class I and class II regions in humans (HLA) and mice (H-2) [reviewed in (2)]. These class III proteins include the serum complement components C4, C2, and factor B (Bf). Complement component C4 is a glycoprotein (200 kd) composed of three disulfide-linked polypeptide chains α, β, and γ. Both C2 (102 kd) and Bf (90 kd) are single-chain glycoproteins with unusual serine protease activity. The class III complement proteins activate C3 either by the classical (C4, C2) or the alternative (Bf) complement pathways. In the classical pathway, activation of C4 leads to the release of a C4a peptide from the N-terminal α chain. Activation of C2 produces two noncovalently linked fragments, C2b and C2a. In the alternative pathway, Bf is cleaved to yield the N-terminal Ba fragment and the C-terminal Bb fragment.
Extensive molecular studies on the human and mouse genomes have resulted in the isolation, characterization, and orientation of the class III genes (3, 4). In man, the HLA class III genes are arranged as shown in Figure 1A (4, 5). The single C2 and Bf loci, which are less than 0.5 kb apart, probably arose by a gene duplication event. Two closely linked C4 loci, C4A and C4B, are about 30 kb apart from the C2-Bf region. Two 21-hydroxylase (21-OHase) genes have also been mapped 3' to both C4A and C4B (8, 9). A similar organization of class III and 21-OHase genes has been reported for the mouse H-2S region (10).

This paper describes the use of human C2 and Bf specific cDNA probes to analyze the C2 and Bf genes in inbred and recombinant miniature swine. The miniature swine used for this study have been inbred for swine MHC (SLA) haplotypes a, c, and d (11) and have been bred as a model for organ transplantation studies (12). These animals have also been particularly useful in immune response and disease resistance studies [reviewed in (13)]. Former studies on the molecular analysis of SLA genes from miniature swine have been focused on the analysis of the class I and class II genes, including the cloning and characterization of the class I genes (14, 15) and the RFLP analysis of the class II genes (13). The class I genes have been isolated from a d haplotype genomic library by screening with a human class I cDNA probe (14). Sequence
Figure 1. Schematic diagram illustrating: (A) Molecular map of human class III genes in the HLA complex [from Ref. (5)]. (B) Organization of human C2 mRNA, its encoded polypeptide and representation of encoding region of the human C2 probe. The pC201 was double digested with ClaI and BamHI to give the single probe shown [from Ref. (6)]. (C) Organization of Bf mRNA, its encoded polypeptide and representation of the encoding regions of the Ba and Bb probes. The pFB3b probe was double digested with ClaI and BamHI to give the two probes shown. In this illustration, the whole Ba region is reversed with respect to Bb because an inversion of this region occurred during the cDNA cloning [from Ref. (7)].
A

HLA Class III genes

[Diagram of chromosome 6 showing the location of HLA Class III genes, including C2, Factor B, C4, and 21-OHase.]

B

C2

[Diagram of C2 showing C2a and C2b regions, with mRNA and probe information.] C2 probe (pC201) [0.4 kb]

C

Factor B

[Diagram of Factor B showing Ba and Bb regions, with mRNA and probe information.] Factor B probes (pFB3b) [0.66 kb, 1.7 kb]
homology analysis of the swine class I genomic clones relative to other species indicates that swine class I genes are most homologous to human class I genes, next most homologous to rabbit class I genes and least homologous to mouse class I genes (15). Swine class I genes have also been mapped to chromosome 7 by direct in situ hybridization (16, 17). Southern blot analysis of the swine class II genes using human class II cDNA probes indicates that the SLA class II region probably is similar to the HLA class II region (13). Because these inbred and recombinant miniature swine are useful for biomedical research, it is of interest to study the organization of the SLA complex. We report here the molecular analysis of the swine class III genes, C2 and Bf.
MATERIALS AND METHODS

Animals

Miniature swine of three SLA haplotypes (SLA a, c, and d) and one intra-SLA recombinant line (SLA g), developed by Sachs et al. (11) and Pennington et al. (18), were brought to Iowa State University in 1982 from the NIH Animal Center. All the inbred and recombinant miniature swine used in this study were bred and maintained at the Bilsland Swine Breeding Farm owned by Iowa State University. The SLA haplotypes of bred miniature swine were serologically determined by using a microcytotoxicity assay as previously described (19). Swine of the a, c, d, and g haplotypes were used for these studies. In addition, a family of five animals, an SLA a/d sire and an SLA a/d dam with three offspring, one SLA a/d and two SLA d/d, was also studied. This family was selected for the analysis of gene segregation. Besides the miniature swine, six unrelated human volunteers were used to generate human genomic DNA controls.

DNA Isolation

High molecular weight genomic DNA was isolated from white blood cells. Briefly, cells were collected from 10 ml of peripheral blood after treatment with erythrocyte lysis solution [0.83% NH4Cl, 0.1 mM ethylenediamine tetraacetate-Na2 (EDTA), 10 mM KHCO3, pH 7.2] twice at 4°C for 5 min each. The
white cells were incubated with 10 ml of lytic solution [10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.6% sodium dodecyl sulfate (SDS)] in the presence of 100 µg/ml ribonuclease A (Boehringer Mannheim, Indianapolis, IN) for 1 h at 37°C. The samples were then digested with proteinase K (500 µg/ml) (Boehringer Mannheim) overnight at 37°C. DNA was extracted gently once with an equal volume of 0.1 M Tris-HCl buffer (pH 8.0) equilibrated phenol, twice with an equal volume of phenol-chloroform-isoamyl alcohol (1:1:0.04 v/v/v), and twice with an equal volume of chloroform-isoamyl alcohol (1:0.04 v/v). High molecular weight DNA was then spooled in 70% ethanol with 100 mM Na acetate, pH 5.2, dried and redissolved in 1 ml of sterile distilled water. Yields varied from 100 µg to 500 µg DNA per 10 ml of peripheral blood.

Restriction Endonuclease Digestion

Ten micrograms of genomic DNA samples were digested twice, with 4 or 10 units/µg of restriction endonucleases (Bethesda Research Laboratories, Gaithersburg, MD), for 2-3 h at conditions recommended by the manufacturer. Digestions of genomic DNA with EcoRI (10 units/µg), BamHI (10 units/µg), HindIII (10 units/µg), PvuII (4 units/µg), BglII (10 units/µg), and SstI (4 units/µg) were performed at 37°C. Digestion with TaqI (4 units/µg) was performed at 65°C. HindIII digested lambda-phage DNA fragments (New England Biolabs, Beverly, MA) were used as DNA size markers.
Southern Blot Analysis

Ten micrograms of digested DNA samples were subjected to electrophoresis in 0.8% agarose (Sigma, St. Louis, MO) gels for 16-24 h at 2 V/cm. The digested DNA fragments in the gels were denatured in 0.5 M NaOH/1.5 M NaCl for 1 h, neutralized with 3 M NaCl/1 M Tris-HCl, pH 5.5, for 1 h, and then transferred to nitrocellulose filters (Schleicher and Schuell, Keene, NH) by the procedure of Southern (20). The nitrocellulose filters were baked under vacuum at 80°C for 2 h. The filters were prehybridized at 42°C for 8-18 h in a solution of 50% (v/v) formamide, 1 M NaCl, 10% dextran sulfate, 10X Denhardt's solution, 1% SDS, 0.1% Na pyrophosphate, 50 mM Tris-HCl, pH 7.5, and 200 µg/ml of sheared, heat-denatured salmon sperm DNA. Hybridization was performed overnight at 42°C with $^{32}$P-labeled, heat-denatured cDNA probes (1x10$^6$ cpm/ml) in the same solution without NaCl. Hybridized filters were washed three times at room temperature in 2X SSC (1X SSC = 0.15 M NaCl, 15 mM Na citrate, pH 7.0), 0.1% SDS for 10 min each, and three times at 52°C in 0.1X SSC, 0.1% SDS for 10 min each. Autoradiograms were obtained by exposing Kodak XAR-5 films to the dried filters with Dupont Lightning Plus intensifying screens at -70°C for 2-8 days.

cDNA Probes

Three HLA class III cDNA probes (6, 7), designated C2, Ba, and Bb (see Fig. 1B and 1C), were used in the Southern blot
analysis to identify C2 and Bf restriction fragments in swine. The C2 probe used in this study was excised with CalI and BamHI from a cDNA clone pC201 generously provided by Dr. David R. Bentley, Department of Biochemistry, Oxford University, Oxford, UK (6). This cDNA insert is about 0.4 kb and encodes a short region of the human C2 protein near its carboxyl-terminus (see Fig. 1B). Two probes, Ba and Bb, were used in the study of Bf DNA polymorphism. Both probes were derived from the pFB3b cDNA clone, which was generously provided by Dr. R. Duncan Campbell, Department of Biochemistry, Oxford University, Oxford, UK (27). The pFB3b clone contains a sequence about 2.3 kb which encodes the mature Bf protein. After BamHI/Clal digestion, two cDNA insert fragments of 0.66 kb and 1.6 kb were produced as a result of an internal BamHI site in the insert. The 0.66 kb fragment is Ba specific and encodes most of the human Ba sequence, whereas the 1.6 kb fragment encodes the whole Bb sequence, a short region of the Ba gene, and the 3'-untranslated region of the mRNA (see Fig. 1C). After the BamHI and Clal double digestion, the C2, Ba and Bb cDNA inserts were isolated from the plasmid vectors by electroelution (21). These cDNA inserts were then radio-labeled using $^{32}$P-dCTP (New England Nuclear, Boston, MA) by nick translation (21) to specific activities of approximately $3 \times 10^8$ cpm/µg DNA.
RESULTS

Human Controls

To test the stringency of hybridization conditions and to prove the specificity of the probes used in this study, genomic DNA from human individuals was analyzed by Southern blot hybridization with the homologous human probes for C2, Ba, and Bb. For human C2 controls, each DNA sample was digested with BglII, BamHI, SstI, and PvuII. After hybridization, all six individuals produced the same restriction patterns. Results from one individual are shown in Figure 2A. These C2 hybridization results agree with the previously published results by Bentley and Porter (16) and by Carroll et al. (4) (a 5.7 kb BglII fragment, two BamHI fragments of 1.6 kb and 1.7 kb, a 7.5 kb SstI fragment, and an 8.4 kb PvuII fragment). For human Bf controls, each genomic DNA sample was digested with PvuII and HindIII and hybridized with the Ba and Bb probes. The results are shown in Figures 2B and 2C. The PvuII pattern agrees with the published result of an 8.4 kb fragment (4). Separate data on the Ba and Bb probes have not been published, but it has been shown that the mixture of Ba and Bb probes from the pFB3b cDNA clone hybridizes to 4.4 kb and 2.7 kb fragments after HindIII digestion (4, 7). Our HindIII restriction patterns (see Figures 2B and 2C) show that the Ba probe hybridizes more strongly to the smaller size band (2.5 kb), whereas the Bb
Figure 2. Southern blot analysis of restriction enzyme digested human genomic DNA by using the following human cDNA probes: (A) the 0.4 kb C2 probe; (B) the 0.66 kb Ba probe; and (C) the 1.6 kb Bb probe. HLA haplotype of this individual is unknown. The restriction endonuclease used is indicated above each track (B: BglII; M: BamHI; S: SstI; P: PvuII; H: HindIII). The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments.
probe hybridizes more strongly to the larger size band (4.6 kb). These results are expected because there is a HindIII site within the Ba sequence (7). After HindIII digestion, most of the Ba sequence is found in the 2.7 (2.5 from our data) kb fragment and part of the Ba sequence is found in the 4.4 (4.6 from our data) kb fragment. Since the Ba probe encodes a large portion of the Ba sequence, it hybridized strongly to the smaller restriction fragment and weakly to the larger size fragment (Fig. 2A, lane 2). The Bb probe encodes a short sequence of the Ba, so the Bb probe hybridizes not only to the larger fragment but also weakly to the smaller Ba fragment (Fig. 2C, lane 2).

RFLP Analysis Using the Human C2 Probe

Genomic DNA samples from SLA a/a, c/c, and d/d miniature swine were digested with six different restriction enzymes (EcoRI, BamHI, HindIII, PvuII, BglII, and TaqI) and analyzed by Southern blot hybridization using the 0.4 kb human C2 probe. With this probe, no polymorphisms were detected in these three SLA inbred lines (see Fig. 3). The C2 probe hybridized to a single EcoRI fragment, a single BamHI fragment, a single HindIII fragment, two PvuII fragments, a single BglII fragment, and a single TaqI fragment.
Figure 3. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA by using the 0.4 kb human C2 probe. The restriction endonucleases used are as indicated: (A) EcoRI; (B) BamHI; (C) HindIII; (D) PvuII; (E) BglII; and (F) TaqI. SLA haplotype corresponding to the DNA sample is indicated below each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments.
SLA haplotype a c d

A
EcoRI

1 2 3

(kb)

B
BamHI

1 2 3

(kb)

C
HindIII

1 2 3

(kb)

5.5

1.2

5.9
Figure 3. Continued
RFLP Analysis Using the Human Ba Probe

Southern hybridization analysis was performed on restriction enzyme digested swine genomic DNA by using the human Ba probe. As shown in Figure 4A-E, there were no restriction fragment polymorphisms in SLA a, c, and d animals when enzymes EcoRI, BamHI, HindIII, PvuII, and BglIII, were used. When TaqI was used, however, three polymorphic restriction fragments and a common fragment were identified in the three SLA inbred lines. As shown in Figure 4F, the Ba probe hybridized to three fragments (1.4 kb, 1.1 kb, and 0.6 kb) in the SLA a and c haplotypes and two TaqI fragments (1.7 kb and 1.4 kb) in the d haplotype. The observed restriction patterns suggest that the 1.7 kb is derived from the 1.1 kb and 0.6 kb fragments. The TaqI polymorphism detected would then correspond to the presence or absence of a single TaqI site in the swine Ba region. Loss of a TaqI site would result in the occurrence of the 1.7 kb fragment.

A family consisting of an a/d sire, an a/d dam and three offspring, d/d, d/d, and a/d, was used in a segregation analysis of the TaqI polymorphism. The parents, having the a/d heterozygous SLA haplotype, presented a composite TaqI RFLP pattern of the a/a and the d/d homozygotes (Fig. 5, lanes 1 and 2). In the offspring, the d/d homozygous animals (Fig. 5, lanes 3 and 4) inherited a d allele from each parent and presented a TaqI RFLP pattern identical to SLA d pigs (Fig.
Figure 4. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA by using the 0.66 kb human Ba probe. The restriction endonucleases used are as indicated: (A) EcoRI; (B) BamHI; (C) HindIII; (D) PvuII; (E) BglII; and (F) TaqI. SLA haplotype of the DNA sample is indicated below each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments.
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>BamHI</td>
<td>HindIII</td>
</tr>
<tr>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
</tbody>
</table>

(kb) (kb) (kb)

5.6

5.8

2.7

1.4

SLA haplotype

a c d

a c d

a c d
Figure 4. Continued
Figure 5. Southern blot analysis of TagI digested miniature swine genomic DNA of a family (SLA a/d sire, SLA a/d dam and three offspring: two SLA d/d and one SLA a/d) by using the 0.66 kb human 8a probe. SLA haplotype corresponding to the DNA sample is indicated under each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments.
TaqI

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

(kb)

1.7
1.4
1.1
0.6

SLA haplotype: a/d a/d d/d d/d a/d
(♂) (♀)
4F, lane 1). The a/d offspring (Fig. 5, lane 5) codominately inherited all the polymorphic TaqI fragments from the parents.

**RFLP Analysis Using the Bb Probe**

Analysis of restriction digests of genomic DNA from SLA inbred lines by using the 1.6 kb Bb probe showed that there were no RFLPs detected when enzymes EcoRI, BamHI, HindIII, PvuII, and BglII were used (Fig. 6A-E). As in the Ba hybridization, the Bb probe produced two TaqI RFLP patterns in the three inbred lines. The a and c haplotypes were associated with one RFLP pattern including four restriction fragments, 1.5 kb, 1.3 kb, 1.1 kb, and 1.0 kb, while the d haplotype was associated with the other pattern consisting of three common fragments, 1.5 kb, 1.3 kb, and 1.1 kb (Fig. 6F).

The same a/d x a/d family was studied for the segregation patterns of the TaqI polymorphism. Figure 7 shows both a/d parents and the a/d offspring contained the 1.0 kb TaqI polymorphic fragment. Each of the two d/d offspring showed only the three common fragments from the d allele of each parent. This Mendelian inheritance pattern confirms the association of the 1.0 kb fragment with the a haplotype. We cannot exclude the possibility that this polymorphism has resulted from the same TaqI polymorphic site detected with the Ba probe because the Bb probe we used hybridizes to part of the Ba sequence.
Figure 6. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA by using the 1.6 kb human Bb probe. The restriction endonucleases used are as indicated: (A) EcoRI; (B) BamHI; (C) HindIII; (D) PvuII; (E) BglII; and (F) TaqI. SLA haplotype corresponding to the DNA sample is indicated below each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments. ND: not determined; the size of the fragment was not estimated (>23 kb).
A

EcoRI

1 2 3

B

BamHI

1 2 3

C

HindIII

1 2 3

ND (kb)

---

5.5

---

5.0

---

3.8

---

5.8

---

3.7

---

2.7

---

2.3

---

1.3

---

1.2

SLA haplotype

a c d a c d a c d
Figure 6. Continued
Figure 7. Southern blot analysis of TaqI digested miniature swine genomic DNA of a family (SLA a/d sire, SLA a/d dam and three offspring: two SLA d/d and one SLA a/d) by using the 1.6 kb human Bβ probe. SLA haplotype corresponding to the DNA sample is indicated below each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments. The association of the 1.0 kb polymorphic fragment with the Α allele is demonstrated in this segregation analysis.
TaqI

1 2 3 4 5

(kb)

---

SLA haplotype a/d a/d d/d d/d a/d

(♂) (♀)
The Close Linkage of the C2 and Bf Genes

A comparison of the restriction patterns obtained with the C2 and the Ba probes suggested that common EcoRI (5.5 kb vs. 5.6 kb), BamHI (1.2 kb vs. 1.4 kb), HindIII (5.9 kb vs. 5.8 kb), PvuII (6.3 kb vs. 6.3 kb), BglII (3.7 kb vs. 3.5 kb), and TaqI (1.4 kb vs. 1.4 kb) restriction fragments were identified with either probe. To confirm that the common fragments were indeed the same sizes, duplicate sets of EcoRI, HindIII and PvuII digested DNA from a single animal were run on the same gel. The sets were then hybridized with the C2 and Ba probes respectively. Figure 8 shows that the restriction patterns of both sets are similar. These overlapping fragments were also confirmed by hybridizing a single blot simultaneously with both C2 and Ba probes (data not shown).

Mapping of the Recombinant Haplotype SLA £

The SLA haplotype £ is derived from a recombination event that took place between the class I and class II regions and is characterized as DQ\^\text{d}DR\^\text{d}B\text{c}A\text{c}. Inasmuch as we have shown that the TaqI polymorphism can be used to distinguish the \text{c} allele and the \text{d} allele, analysis of the recombinant £ is interesting. Figure 9 shows that the TaqI RFLP pattern of the £ recombinant is identical to the pattern of haplotype \text{d}, using either the Ba probe (Fig. 9A) or the Bb probe (Fig. 9B). This result indicates that the £ recombinant has a crossover site between the class II-Bf and class I loci. It also
Figure 8. A comparison of EcoRI, HindIII and PvuII restriction patterns in miniature swine with the following two probes: (A) the 0.4 kb human C2 probe and (B) the 0.66 kb human Ba probe. A DNA sample from SLA haplotype d is used in both blots. The restriction endonuclease used is indicated above each track (E: EcoRI; H: HindIII; P: PvuII). The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments. Common restriction fragments are shown by hybridization with both C2 and Ba probes.
Figure 9. Southern blot analysis of TaqI digested miniature swine genomic DNA from SLA \( \alpha \) and SLA \( \delta \) inbreds and from SLA \( g \) recombinant by using the following two probes: (A) the 0.66 kb human Ba probe and (B) the 1.6 kb human Bb probe. SLA haplotype corresponding to the DNA sample is indicated below each track. The RFLP pattern of the intra-SLA recombinant haplotype \( g \) is identical to the pattern of haplotype \( \delta \).
implies that the Bf-C2 region is positioned either centromeric to the class II region or between the class II and class I regions.
DISCUSSION

The data in this paper show that a TaqI restriction fragment length polymorphism is detectable in the SLA complex with the use of a human Bf probe. This new SLA marker allows haplotype d to be distinguished from haplotypes a and c. Since polymorphic variants of human C2 and Bf have been linked to the susceptibility of individuals to disease (22), this TaqI DNA polymorphism in swine may serve as a useful marker in the genetic analysis of SLA-associated physiological and pathological traits.

No DNA polymorphism in swine was detected by using the 0.4 kb human C2 probe. Possible explanations of the failure to detect any polymorphisms are: (1) No C2 DNA polymorphisms exist in these SLA inbred animals; (2) there are RFLPs but they cannot be demonstrated by the six endonucleases we used; or (3) there are RFLPs but they cannot be identified by the particular probe we used. Future work using different restriction endonucleases or different probes may help to provide more information on possible C2 polymorphisms. It is not too surprising that no C2 polymorphisms were identified. In the human system, C2 has less protein polymorphism than any other HLA products. In fact, most individuals (94%) are homozygous for a common C2 variant (5). Only three RFLPs have been identified in the human C2 gene by intensive analysis of many Southern hybridizations on different individuals with a
large variety of endonucleases and various probes. These three polymorphisms of human C2, seen upon digestion with SstI, TaqI, and BamHI, were detected by three different probes: a 0.3 kb C2 genomic probe; a 2.3 kb Bf probe; and a 0.9 kb C2 cDNA probe, respectively [reviewed in (5)]. Because C2 is present in low concentrations in the serum, no full length cDNA probe has been developed yet.

Although no C2 DNA polymorphism was found in swine, common fragments were detected by both the C2 and Bf probes. Inasmuch as the C2 probe used is specific for a region near the 3' end of C2, and the Bf probe is specific to the 5' end of Bf, it is reasonable to suggest that C2 and Bf are closely linked in swine. The isolation of overlapping swine class III genomic clones would confirm this close linkage. Close linkage between C2 and Bf has been found in humans where the two genes are only 425 bp apart. The results in this paper also suggest that swine, as well as humans, have single C2 and Bf genes.

The segregation of the TaqI DNA polymorphism with class II genes, but not the class I genes in the recombinant haplotype g, shows that the crossover point of the g haplotype must be positioned between the class II-Bf and class I loci. We do not have direct evidence whether the C2-Bf region is located between the class I and class II regions or centromeric to the class II genes. In the human system, class III C4 genes have
been located between the class I and class II regions by analyzing C4 RFLPs in mutant cell lines with deleted HLA loci (23) and by analyzing the inheritance of C4 RFLPs in recombinants between HLA-DR and HLA-B regions (24, 25). As an example of the analysis of intra-HLA recombinants, Robinson et al. (24) have shown that in the study of five informative families, C4 genes segregated with HLA-DR in four cases and with HLA-B in one. Therefore, analysis of more intra-SLA recombinants, e.g., SLA haplotype f (18), may provide more information on this issue. However, given our results from the g recombinant and the fact that the class III genes lie between class I and class II in the human and mouse, it is reasonable to suggest that swine have the same MHC organization, i.e., the Bf and C2 loci lie between the SLA class I and class II regions. Furthermore, the orientation of human class III genes with respect to the class I and class II loci is unknown, although it has been suggested that C4 is closest to HLA-B and Bf is closest to HLA-DR based on examination of the distribution of linkage disequilibrium in existing HLA haplotypes (26). In the human system, no informative recombination events have been observed within the class III region, nor have overlapping cosmid clones been detected that link the HLA class III with class I or class II genes so far. Confirmation of the order of the genes in the SLA complex awaits results of Southern blot analysis of
pulse-field gel electrophoresis of large fragments of DNA (27, 28).

Linkage of class III molecules to the MHC has also been shown in mammals other than humans and mice by use of protein variants; e.g., C2, Bf, and C4 in guinea pigs, Bf in rhesus monkeys, and Bf in chimpanzees (29-31). The recent availability of cDNA probes has allowed the study of class III polymorphisms to be extended from the protein level to the level of the genes. Our study is the first report that describes the swine C2 gene. A previous report by Vaiman et al. (32) failed to detect swine Bf DNA polymorphisms in French outbred animals by using the human Bf probe FBI, which contains a 515 bp cDNA insert encoding only part of the Bb sequence. However, only one restriction enzyme, EcoRI, was used in their study, which resulted in a single monomorphic restriction fragment of 3.5 kb in the six different SLA haplotypes tested. We believe the 3.5 kb fragment they reported is probably identical to one of the monomorphic EcoRI fragments, 3.7 kb, described in this report.

In conclusion, we have successfully identified swine C2 and Bf restriction fragment patterns in SLA inbred and recombinant lines of miniature swine by Southern blot hybridization with human cDNA probes. The first demonstration of a DNA polymorphism in the swine Bf region is reported in this paper. Our results also indicate a close linkage of the
swine C2 and Bf genes. Finally, the C2-Bf region is linked to the class II genes, as shown by analysis of a recombinant haplotype. It is hypothesized that swine C2-Bf is located between the class II and class I genes, as has been demonstrated in mouse and man.
REFERENCES


SECTION III. MOLECULAR ANALYSIS OF SWINE CLASS III GENES. II. MAPPING OF C4 GENE(S) TO THE SWINE MAJOR HISTOCOMPATIBILITY COMPLEX (SLA)
MOLECULAR ANALYSIS OF SWINE CLASS III GENES. II. MAPPING OF C4 GENE(S) TO THE SWINE MAJOR HISTOCOMPATIBILITY COMPLEX (SLA)

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Abbreviations used in this paper: Bf, complement factor B; C2, second component of complement; C4, fourth component of complement; MHC, major histocompatibility complex; 21-OHase, 21-hydroxylase; RFLP, restriction fragment length polymorphism; SLA, swine leukocyte antigen.

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ABSTRACT

Two cDNA probes for the human complement component C4 (C4β-α and C4α-γ) were used to search for restriction fragment length polymorphisms (RFLPs) of swine C4 gene(s). Three miniature swine lines, inbred for SLA haplotypes a, c, and d, and a recombinant line, haplotype g, were used. Data collected with six different restriction enzymes (EcoRI, HindIII, BamHI, BglII, PvuII, and SstI) showed DNA polymorphisms of C4 with three of the enzymes: BamHI, PvuII, and SstI. Segregation analyses on an informative swine family indicated that all the polymorphic fragments detected followed a Mendelian pattern of inheritance. When either BamHI or SstI were used, SLA haplotype a presented a RFLP pattern which was different from the pattern of haplotypes c and d. With PvuII, however, three different RFLP patterns were identified for the three SLA inbred lines. The recombinant haplotype g, which has been characterized as DQ<sup>d</sup>DR<sup>d</sup>(C2-Bf<sup>d</sup>)B<sup>C</sup>A<sup>C</sup>, was shown to produce an identical PvuII RFLP pattern to haplotype d, but different from that of haplotype c. This indicates that C4 is located centromeric to of the class I region of the SLA complex. A tentative plan for SLA gene organization can be shown as DQDR[(C2-Bf)(C4)]BA. The order of C2-Bf-C4 remains to be determined.
INTRODUCTION

Three components of complement, C2, factor B (Bf), and C4, are encoded by closely linked genes in the major histocompatibility complex (MHC) of man (HLA) and mouse (H-2), and are designated as class III genes [reviewed in (1)]. In the HLA complex, these genes are located between HLA-B (class I) and HLA-D (class II) regions and have the order C2, Bf, C4A, and C4B (2). A similar organization of complement genes, C2, Bf, C4, and Sip (sex-limited protein) has been shown in the H-2 complex (3). The Sip in mouse is a hemolytically inactive homolog of C4 with unknown function. White et al. (4, 5) have also mapped two steroid 21-hydroxylase genes, 21-OHaseA and 21-OHaseB, to the MHC of both mouse and man. Each 21-OHase gene lies 3' to each C4 (or Sip) gene (5, 6).

Complement component C4 is a central protein in the activation of the classical complement pathway. It is a serum glycoprotein of approximately 200 kd which is composed of three disulfide-linked polypeptide chains α, β, and γ with molecular weights of approximately 95 kd, 75 kd, and 30 kd, respectively. The C4 protein is synthesized as a single-chain precursor, pro-C4, in the order NH₂-β-α-γ-COOH and then processed to mature C4. A diagram of mature human C4 is shown in Figure 1A (7). Activation of C4 releases a 7 kd C4a peptide from the N-terminal end of the α chain and also...
Figure 1. Schematic diagrams illustrating the organization of (A) mature human C4 protein [from Ref. (7)] and (B) human pro-C4B protein, C4B mRNA, and the human C4α-α and C4α-γ probes. The two probes shown were obtained from the human cDNA clone pAT-F by a ClaI/BamHI double digestion [Ref. (8, 9)].
Mature Human C4

A

\[ \alpha_{\text{NH}_2} \quad \text{C4a} \quad \text{C4d} \quad \text{COOH} \]

\[ \beta_{\text{NH}_2} \quad \text{SS} \quad \text{COOH} \]

\[ \gamma_{\text{NH}_2} \quad \text{SS} \quad \text{COOH} \]
Figure 1. Continued
B

Human C4B

NH₂ β α γ COOH pro-C4B protein

β α γ (≈2100 bp) (≈2300 bp) (≈900 bp) C4B mRNA

C4 probes

C4β-α (2.5 kb) C4α-γ (2.3 kb) (pAT-F)
exposes a reactive acyl group in the C4d region of the α chain.

The observed polymorphisms of C4 in man are greater than those for the C2 and Bf genes. There are two forms of human C4, C4A and C4B, which can be distinguished by electrophoresis (10) and by specific antisera, anti-Rodgers (C4A) and anti-Chido (C4B) (11). Functionally, these two isotypes have different hemolytic activities (12). At the DNA level, a comparison of the sequence of more than 4.6 kb of cDNA from C4A and C4B showed that the major differences detected between the two genes are 15 nucleotide differences in which 13 are localized in the C4d region (13). The C4A and C4B genes have similar restriction maps, except that a 6 to 7 kb intron is missing from the 5' region in some C4B alleles (14, 15). Both C4A and C4B loci are highly polymorphic. Allelic differences have been recognized at the protein level by electrophoresis and serology (16) and at the DNA level by RFLP analysis (17, 18) and nucleotide sequencing (13). Most of the differences among C4 alleles have been shown to be in the C4d region. In addition to the isotypic differences and allelic variants, gene duplications, null alleles, or total deficiency of human C4 have also been described [reviewed in (15)]. It has been suggested that the high degree of polymorphism of C4 may be biologically relevant to ensure interaction with a wide range of pathogens (19). In certain circumstances, the C4
polymorphism may also affect the relative risk for a number of autoimmune diseases (20).

We are interested in MHC gene organization of the SLA complex of inbred miniature swine (21). These animals have provided a good model for studies of organ transplantation (22) and SLA associated immune responses (23). The molecular analysis of class I and class II genes in these miniature swine has shown that there is high homology between the SLA complex and the HLA complex [reviewed in (23)]. However, less is known about the SLA class III genes. Mapping of C4 EcoRI restriction fragments to the SLA complex has been demonstrated in French outbred swine by Kirszenbaum et al. (24). Recently, using SLA inbred and recombinant miniature swine as a model, we have successfully mapped the swine C2 and Bf genes to the SLA complex (25). In this paper, we continue the RFLP analysis of swine class III genes, namely, an analysis of the C4 genes in miniature swine.
MATERIALS AND METHODS

Animals

Miniature swine of three SLA haplotypes (SLA a, c, and d) and one intra-SLA recombinant line (SLA g), developed by Sachs et al. (21) and Pennington et al. (26), were brought to Iowa State University in 1982 from the NIH Animal Center. All the inbred and recombinant miniature swine used in this study were bred and maintained at the Iowa State University Bilsland Swine Breeding Farm at Madrid, Iowa. The SLA haplotypes of these miniature swine were serologically determined, by using a microcytotoxicity assay, as previously described (27). Swine of the a, c, d, and g haplotypes were used for these studies. In addition, a family of four animals, an SLA a/a sire and an SLA c/c dam with two heterozygous SLA a/c offspring, was also studied. This family was selected for the analysis of gene segregation. In addition to the miniature swine, five unrelated human volunteers were used to generate human genomic DNA controls.

DNA Isolation and Restriction Endonuclease Digestion

High molecular weight genomic DNA was prepared from peripheral white blood cells according to the method described previously (25). Ten micrograms of genomic DNA samples were digested twice, with restriction endonucleases (Bethesda Research Laboratories, Gaitherburg, MD), EcoRI (10 units/μg),
BamHI (10 units/µg), HindIII (10 units/µg), PvuII (4 units/µg), BglII (10 units/µg), and SstI (4 units/µg), for 2-3 h at conditions recommended by the manufacturer. HindIII digested lambda-phage DNA fragments (New England Biolabs, Beverly, MA) were used as DNA size markers.

Southern Blot Analysis

The restriction fragments were separated by agarose gel electrophoresis (0.8%), denatured, neutralized and blotted onto nitrocellulose filters by the standard method (28). Prehybridization, hybridization, and autoradiography were as described previously (25).

cDNA Probes

Two human C4 cDNA probes (see Fig. 1B), designated C4β-α and C4α-γ, were used in the Southern blot analyses to identify C4 restriction fragments in swine. Both probes were derived from the pAT-F clone (8, 9), which was generously provided by Dr. Michael C. Carroll, Department of Pediatrics, Harvard Medical School, Children's Hospital, Boston, MA. The pAT-F clone contains a 4.8 kb insert that encodes most of the β chain, the complete α chain, and the complete γ chain on the human pro-C4B protein. After BamHI/ClalI digestion of pAT-F, two cDNA insert fragments of 2.5 kb and 2.3 kb were isolated from the plasmid vector by electroelution (29). The 2.5 kb C4β-α probe encodes the 5' region of pro-C4B, whereas the 2.3
kb C4α-γ probe encodes the 3' region of pro-C4B. These two cDNA inserts were radiolabeled using $^{32}$P-dCTP (New England Nuclear, Boston, MA) by nick translation (29) to specific activities of approximately $3 \times 10^8$ cpm/µg DNA.
RESULTS

Human Controls

Genomic DNA samples from five unrelated human volunteers were analyzed by Southern blot hybridization using the human C4β-α and C4α-γ probes. In this experiment, four restriction enzymes, EcoRI, HindIII, BamHI, and BglII, were used. Figures 2A-E show the results from the analysis of DNA from five individuals hybridized with the C4β-α probe. With the C4β-α probe, three (Fig. 2C, 2D, and 2E) of the five individuals had identical RFLP patterns with all four enzymes. One individual (Fig. 2A) had polymorphic EcoRI, HindIII, and BamHI patterns, and another individual (Fig. 2B) had another polymorphic BamHI pattern. With use of the C4α-γ probe, however, all five individuals had identical restriction patterns. The results from one individual are shown in Figure 3. The C4 protein types of these human volunteers are unknown.

Swine RFLP Analysis Using the Human C4β-α Probe

Genomic DNA samples from SLA a/a, c/c, and d/d miniature swine were digested with five different restriction enzymes (EcoRI, BamHI, HindIII, PvuII, and BglII) and analyzed by Southern blot hybridization using the 2.5 kb human C4β-α probe. As shown in Figures 4A, 4C, and 4E, there were no restriction fragment polymorphisms when enzymes EcoRI, HindIII, and BglII were used. With BamHI and PvuII, however,
Figure 2. Southern blot analysis of restriction enzyme digested human genomic DNA by using the human C4β-α probe. Autoradiograms of the five unrelated individuals are shown as A, B, C, D, and E, respectively. The restriction endonuclease used is indicated above each track (E: EcoRI; H: HindIII; M: BamHI; B: BglII). Size estimations were made from HindIII digested lambda-phage DNA fragments.
Figure 2. Continued
Figure 3. Southern blot analysis of restriction enzyme digested human genomic DNA by using the human C4α-γ probe. The restriction endonuclease used is indicated above each track (E: EcoRI; H: HindIII; M: BamHI; B: BglII). Size estimations were made from HindIII digested lambda-phage DNA.
Figure 4. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA from three SLA inbred lines by using the human C4β-α probe. The restriction endonucleases used are as indicated: (A) EcoRI; (B) BamHI; (C) HindIII; (D) PvuII; (E) BglII. SLA haplotype of the DNA sample is indicated below each track. The sizes of restriction fragments were estimated from HindIII digested lambda-phage DNA. ND: Not Determined (too large, >23 kb).
<table>
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<th>A</th>
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<tr>
<td>EcoRI</td>
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<td>1.8</td>
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SLA haplotype:

a c d  a c d  a c d  a c d
Figure 4. Continued
polymorphic restriction fragments were observed in the three SLA inbred lines (Fig. 4B and 4D). With BamHI, there were two polymorphic fragments, 2.1 kb and 2.0 kb, associated with haplotype a (2.1 kb) and haplotypes c and d (2.0 kb), respectively (Fig. 4B). With PvuII, as shown in Figure 4D, three different RFLP patterns were detected among the three SLA haplotypes. A total of six polymorphic fragments were observed: 1.4 kb in c, 1.3 kb in a and d, 1.1 kb in a, 1.0 kb in c and d, 0.6 kb in c and d, and 0.5 kb in a.

A family consisting of an a/a sire, a c/c dam, and two heterozygous a/c offspring was used in the segregation analyses of the BamHI and PvuII polymorphisms. With BamHI, as shown in Figure 5A, the a/c heterozygotes codominantly inherited the 2.1 kb and 2.0 kb fragments from the a/a and c/c parents. With PvuII, as shown in Figure 5B, the a/c offspring had all six polymorphic fragments and represent a composite PvuII pattern of the homozygous parents.

Swine RFLP Analysis Using the C4α-γ Probe

The RFLP analyses of genomic DNA from SLA inbred lines, using the C4α-γ probe, were performed with six different restriction enzymes (EcoRI, BamHI, HindIII, PvuII, BglII, and SstI). No polymorphisms were detected when EcoRI, BamHI, HindIII, PvuII, and BglII were used (Fig. 6A-E). With SstI, however, two different RFLP patterns were identified in the three SLA inbred lines (Fig. 6F). The a haplotype was
Figure 5. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA of a family (SLA a/a sire, SLA c/c dam, and two SLA a/c offspring) by using the human C48-α probe. Restriction enzymes used are as indicated: (A) BamHI and (B) PvuII. SLA haplotype corresponding to the DNA sample is indicated under each track. The sizes of restriction fragments were estimated on the basis of HindIII digested lambda-phage DNA fragments. The lower intensity of BamHI fragments in the SLA c/c [(A) lane 2] is due to the lower amount of DNA sample loaded. With either BamHI (A) or PvuII (B), the SLA heterozygous offspring (lanes 3 and 4) presented the restriction fragments inherited from both homozygous parents (lane 1 plus lane 2).
A  
BamHI  
1  2  3  4

B  
PvuII  
1  2  3  4

(kb)

-4.8  -3.7
-2.8  -1.7
-2.1  -2.0
-1.8
-1.5  -1.4
-1.3
-1.1  -1.0
-0.8  -0.7
-0.6  -0.5

SLA  
ahalotype  
a/a  c/c  a/c  a/c
(♂)  (♀)

a/a  c/c  a/c  a/c
(♂)  (♀)
Figure 6. Southern blot analysis of restriction enzyme digested miniature swine genomic DNA from three SLA inbred lines by using the human C4a-γ probe. The restriction endonucleases used are as indicated: (A) EcoRI; (B) BamHI; (C) HindIII; (D) PvuII; (E) BglII; and (F) SstI. SLA haplotype corresponding to the DNA sample is indicated below each track. The sizes of restriction fragments were estimated on the basis of HindIII digested lambda-phage DNA fragments. ND: Not Determined (too large, >23 kb).
SLA haplotype a c d a c d a c d
Figure 6. Continued
associated with one RFLP pattern, which contains the 4.2 kb polymorphic fragment. The c and d haplotypes were associated with another pattern, which contains the 1.8 kb polymorphic fragment. The same a/a x c/c family was studied for the segregation patterns of the SstI polymorphism. Figure 7 shows that both a/c offspring inherited the 4.2 kb fragment from the a/a parent and the 1.8 kb fragment from the c/c parent.

Mapping of the Recombinant Haplotype SLA g

The SLA haplotype g is derived from a recombination event that took place between the class I and class II regions. To locate the C4 gene(s) in the SLA complex, RFLP analysis of the g haplotype, using PvuII and the C4g-a probe, was performed. As shown in Figure 8, the PvuII pattern of the g recombinant haplotype is identical to the pattern of the d haplotype, but different from that of the c haplotype. This shows that C4 has segregated with the class II genes in the g recombinant haplotype. This result indicates that C4 is positioned either centromeric to the class II region or between the class II and the class I regions.
Figure 7. Southern blot analysis of SstI digested miniature swine genomic DNA of a family (SLA a/a sire, SLA c/c dam, and two SLA a/c offspring) by using the human C4a-γ probe. SLA haplotype corresponding to the DNA sample is indicated below each track. The sizes of restriction fragments were estimated on the basis of HindIII digested lambda-phage DNA fragments. The lower intensity of fragments in the SLA c/c haplotype (lane 2) is due to the lower amount of DNA sample loaded. The 0.8 kb monomorphic SstI fragment did not show up on this autoradiogram. The SLA heterozygous offspring (lanes 3 and 4) presented the restriction fragments inherited from both homozygous parents (lane 1 plus lane 2).
SstI
1 2 3 4 (kb)

4.2
3.8
2.7
1.8
1.4

SLA haplotype a/a c/c a/c a/c
(♂) (♀)
Figure 8. Southern blot analysis of PvuII digested miniature swine genomic DNA from SLA c and SLA d inbred animals and from a SLA g recombinant animal by using the human C4b-α probe. SLA haplotype corresponding to the DNA sample is indicated below each track. The RFLP pattern of the recombinant haplotype g is identical to the pattern of haplotype d.
DISCUSSION

In this study, swine C4 RFLPs were demonstrated in three SLA inbred lines by using two human C4 cDNA probes and the restriction enzymes BamHI, PvuII, and SstI. These C4 DNA polymorphisms may be useful in the genetic analysis of SLA-associated immune responses and may also serve as additional markers in the genetic analysis of the SLA complex. By analyzing the PvuII RFLP pattern in the intra-SLA recombinant haplotype g, a close association of the C4 and the class II genes was shown. Therefore, swine C4 gene(s) are located in the SLA complex, either centromeric to the class II genes or between the class I and class II genes.

The mapping of swine C4 gene(s) to the SLA complex in French outbred animals has been suggested by Kirszenbaum et al. (24) based on an association of EcoRI restriction fragments to specific SLA haplotypes in nine siblings. In their study, a common 11.5 kb fragment and a polymorphic 12 kb fragment were identified by using a 310 bp human C4d specific cDNA probe. No segregation analyses were performed on these EcoRI fragments. On the basis of their results, Vaiman et al. (30) suggested that there is only one C4 gene in swine.

Our studies do not show unequivocally the number of C4 genes in miniature swine. A comparison of the C4 restriction fragments from human (Fig. 2 and Fig. 3) and swine (Fig. 4 and Fig. 6) shows that both species had similar numbers and sizes.
of HindIII and BglII fragments. However, with EcoRI or HindIII and the C4β-α probe, the human patterns show more fragments and/or some fragments with larger sizes than those from swine. We believe that these differences may be due to: (a) different C4 restriction maps between miniature swine and humans, (b) heterozygosity of the human C4 loci compared with homozygosity of the swine C4 loci, (c) a possible higher C4 gene copy number in the human genome compared to the swine genome, or (d) any combination of these. Further analyses (e.g., molecular cloning and chromosomal walking) are required to establish conclusively the C4 gene number in swine.

The existence of SLA inbred and recombinant lines and the availability of human class III cDNA probes have provided us the opportunity to map the swine C2 and Bf genes to the SLA complex (25). In this study, mapping of the C4 gene(s) to the SLA complex was accomplished. By studying the intra-SLA recombinant line, SLA g, all the swine class III complement genes, C2, Bf and C4, have been demonstrated to be positioned either between the class II region and the class I region or centromeric to the class II region. The former is considered more likely because of known homologies of MHC organization among species. The SLA complex can be tentatively characterized as DQDR[(C2-Bf)(C4)]BA. The exact order and position of C2, Bf, and C4 in the SLA complex remains to be determined.
REFERENCES


SECTION IV. PREPARATION AND CHARACTERIZATION OF MURINE MONOCLONAL ANTIBODIES TO SWINE LYMPHOCYTE ANTIGENS
PREPARATION AND CHARACTERIZATION OF MURINE MONOCLONAL ANTIBODIES TO SWINE LYMPHOCYTE ANTIGENS

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Abbreviations used in this paper: $\beta_2^m$, $\beta_2$-microglobulin; ELISA, enzyme-linked immunosorbent assay; MAb, monoclonal antibody(s); MHC, major histocompatibility complex; PMNC, peripheral blood mononuclear cells; SaCI, Staphylococcus aureus Cowan I; SLA, swine leukocyte antigen.

We thank Dr. Joan Lunney and Dr. Mark Pescovitz for their advice, discussions, and help in collecting preliminary data. We also thank Dr. Joan Lunney for the gifts of purified swine $\beta_2^m$, anti-$\beta_2^m$ serum, and normal goat serum. We thank Dr. David Sachs for the 74-11-10 MAb hybridoma cell line and the anti-SLa$d$ alloantiserum. We thank David Meeker and John Newton for blood sample collection. Technical assistance by Vickie Hall in the flow cytometric analysis is acknowledged.
ABSTRACT

A panel of hybridoma lines was developed by the fusion of Sp2/0 myeloma cells and spleen cells from mice immunized with peripheral blood mononuclear cells (PMNC) or T cells from NIH SLA inbred miniature swine. Twenty stable hybridoma clones were isolated that secreted monoclonal antibodies (MAb) that reacted with swine PMNC, as determined by an enzyme-linked immunosorbent assay (ELISA). The binding profile to swine PMNC and the ability to fix complement of these MAb were investigated by flow cytometric analyses. The MAb bound different percentages of PMNC, but no differences were found among genetically different swine. Four of the 20 MAb were able to fix complement. The molecular weights of the antigens recognized by six of the MAb were determined by immunoprecipitation of ${^{125}}$I surface labeled PMNC, followed by SDS-PAGE under reducing conditions. The most interesting MAb, 7-34-1 (IgG2a), which had reactivity to almost all swine PMNC tested (~98%), precipitated a putative MHC class I molecule composed of a 50 kd heavy chain and a 12 kd light chain ($\beta_2m$). The immunoprecipitate results, as well as the flow cytometric profile of MAb 7-34-1 on PMNC, strongly suggest that MAb 7-34-1 is an anti-SLA class I MAb. Monoclonal antibody 7-34-1 did not bind radiolabeled swine $\beta_2m$ and was not inhibited by the presence of free $\beta_2m$. These data indicate that the antigenic determinant recognized by 7-34-1 is probably on the
heavy chain of the SLA class I molecule. MAb 7-34-1 was compared to a known class I specific MAb, 74-11-10. Properties of MAb 7-34-1 were different from MAb 74-11-10, and were also different from another SLA class I specific MAb that has been described in the literature (PT85). Monoclonal antibody 7-34-1 recognized class I antigens of SLA haplogypes a, c, and d in an equivalent manner. This MAb should be especially useful as a general anti-SLA class I reagent for experiments on NIH miniature swine.
INTRODUCTION

Miniature swine are excellent large laboratory animals for use in biomedical research. Three lines of SLA inbred miniature swine, designated SLA^a, SLA^c, and SLA^d, have been developed by Sachs et al. (1), as an animal model for studies of organ transplantation (2, 3) and SLA associated immune responses [reviewed in (4)]. With these lines, characterization of swine MHC class I and class II molecules (5-8) and cloning of the class I genes (9) have begun. Homology between the SLA complex and the MHC of mice (H-2) and humans (HLA) has been detected at both the protein and DNA levels.

Our laboratory is interested in the study of SLA genes associated with disease resistance (10, 11) and with development and reproduction (12). Identification of the SLA haplotype of pigs requires the use of specific antisera, monoclonal antibodies, or DNA probes. The availability of antisera for SLA typing is limited. Several laboratories have attempted to produce monoclonal antibodies (MAb) for SLA typing. Two anti-SLA-class I MAb have been reported, 74-11-10 (13) and PT85 (14). Because no swine continuous cell lines are available, fusion of mouse myeloma cells with mouse spleen cells post-xenogeneic immunization has been employed to produce hybridomas. This paper reports the production of 20 murine MAb to swine peripheral blood cell surface antigens, including one MAb to SLA class I antigens.
MATERIALS AND METHODS

Animals

Miniature swine of three SLA haplotypes, \( a^{\text{SLA}} \), \( c^{\text{SLA}} \), and \( d^{\text{SLA}} \), originated by Sachs et al. (1) were brought to Iowa State University in 1982. This SLA inbred miniature swine used in this study were bred and maintained at the Iowa State University Bilsland Swine Breeding Farm at Madrid, Iowa. Female BALB/cJ mice were obtained from The Jackson Laboratory (Bar Harbor, Maine).

MAb 74-11-10 and Swine Alloantiserum

The MAb 74-11-10 (IgG2b), was obtained from Dr. David Sachs' laboratory, NIH. The MAb described by Pescovitz et al. (13), is a cytotoxic Ab with specificity for a SLA class I antigen. Specificity of this MAb has been confirmed by reaction with mouse L cells transformed with the SLA class I genes (15). Anti-SLA\(^d\) alloantiserum was also obtained from Dr. David Sachs' laboratory, NIH.

Swine Cell Preparation

Swine peripheral blood mononuclear cells (PMNC) were isolated from heparinized blood from miniature swine by Ficoll-Hypaque gradient centrifugation (16). Nylon wool-nonadherent cells (T cells) were prepared from swine PMNC as described (17). The activated swine PMNC were prepared by incubating \( 1 \times 10^6 \) PMNC per well \( [1 \times 10^6 \text{ cells/ml in RPMI-1640} \).
supplemented with 2 mM glutamine (GIBCO) and 10% heat inactivated fetal calf serum (FCS) (GIBCO)] with 25 μg phytohemagglutinin (PHA-M, GIBCO) in a 24 well tissue culture plate (Costar, Cambridge, MA) at 37°C, 5% CO2 in air for 72 hr. The cultured cells were then washed with PBS (phosphate buffered saline, pH 7.0) three times and collected as activated PMNC.

MAb Production

Cloned hybridomas were derived from fusions between mouse spleen cells and mouse myeloma cells. Briefly, swine PMNC, T cells, or activated PMNC were used as immunogens (Table 1). Female BALB/cJ mice were immunized at least two times i.p. with 2x10^7 swine cells in PBS (see Table 1) on day 0 and day 14. A last boost was done about two weeks later and four days before the fusion except for one experiment in which a total of three booster injections (i.p., i.v., and i.p.) were given on three consecutive days before the fusion (Table 1). Mouse spleen cells (3x10^7 cells) were then fused with the Sp2/0 mouse myeloma cells (3x10^7 cells) by using 45% polyethylene glycol (PEG 1540, Baker). Following the fusions, hybridomas were selected in HAT medium [10^{-2} M hypoxanthine, 5x10^{-7} M aminopterin and 10^{-3} M thymidine in Dulbecco’s modified eagle’s medium (DMEM with high glucose, GIBCO) supplemented with 20% fetal bovine serum (FBS) (Hyclone, Logan, UT), 100 U/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO), and
Table 1. Summary of immunogens, Ig subclasses, and complement fixation activity of a panel of MAb reactive with swine peripheral mononuclear cells

<table>
<thead>
<tr>
<th>MAb</th>
<th>Immunogen</th>
<th>Ig subclass</th>
<th>ELISA</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-5-9</td>
<td>SLA&lt;sup&gt;c&lt;/sup&gt; PMNC</td>
<td>IgG1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7-12-3</td>
<td>SLA&lt;sup&gt;c&lt;/sup&gt; PMNC</td>
<td>IgG2a</td>
<td>+</td>
<td>-</td>
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<tr>
<td>7-26-5</td>
<td>SLA&lt;sup&gt;c&lt;/sup&gt; PMNC</td>
<td>IgG2a</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7-34-1</td>
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<td>IgG2a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7-40-6</td>
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<td>IgG1</td>
<td>+</td>
<td>-</td>
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<td>7-59-7</td>
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<td>IgG1</td>
<td>+</td>
<td>-</td>
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<td>7-77-4</td>
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<td>IgG1</td>
<td>+</td>
<td>-</td>
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<td>7-212-2</td>
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<td>IgG1</td>
<td>+</td>
<td>-</td>
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<tr>
<td>8-8-6</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; Tcells</td>
<td>IgG2a</td>
<td>+</td>
<td>-</td>
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<tr>
<td>8-34-4</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; Tcells</td>
<td>IgG2a</td>
<td>+</td>
<td>+</td>
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<td>IgG2a</td>
<td>+</td>
<td>-</td>
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<tr>
<td>8-115-1</td>
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<td>+</td>
<td>-</td>
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<tr>
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<td>SLA&lt;sup&gt;d&lt;/sup&gt; Tcells</td>
<td>IgG2a</td>
<td>+</td>
<td>-</td>
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<tr>
<td>9-89-3</td>
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<td>IgA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10-14-1</td>
<td>SLA&lt;sup&gt;d&lt;/sup&gt; Activated PMNC</td>
<td>IgM</td>
<td>+</td>
<td>+</td>
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<tr>
<td>12-22-2*</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; PMNC</td>
<td>IgM</td>
<td>+</td>
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<tr>
<td>12-27-3*</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; PMNC</td>
<td>IgM</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>IgG1</td>
<td>+</td>
<td>-</td>
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<tr>
<td>12-47-4*</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; PMNC</td>
<td>IgG1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12-65-3*</td>
<td>SLA&lt;sup&gt;a&lt;/sup&gt; PMNC</td>
<td>IgG1</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*These MAb were produced with three booster injections (i.p., iv., and i.p.) on three consecutive days before the fusion.
50 μg/ml gentamicin (GIBCO)]. Supernatants of growing hybrids were then screened for antibody activity against swine PMNC by using an enzyme-linked immunosorbent assay (ELISA) (described below). ELISA-positive hybrids were then cloned by limiting dilution.

The MAb secreted from ELISA-positive hybridoma clones were collected as culture supernatants or ascites fluids for further characterization. The hybridoma culture supernatants were concentrated 5-20 times (Amicon, Danvers, MA) before use except in the initial ELISA screening, when no concentration was performed. Ascites fluids were produced by injecting 1x10^7 cloned hybridoma cells i.p. into pristane primed BALB/cJ mice. Two MAb, 7-34-1, developed from this study, and 74-11-10, developed by Pescovitz et al. (13), were purified from culture supernatants by ammonium sulfate precipitation followed by protein A-Sepharose (Pharmacia) affinity chromatography (18).

**ELISA**

The ELISA plates were prepared by coating poly-L-lysine (Sigma) (0.01% in PBS) treated, U-bottomed, Immunolon II microtiter plates (Dynatech, Alexandria, VA) with swine cells. Briefly, 2x10^5 PMNC (50 μl of 5x10^6 cells/ml in PBS) were added to each well and allowed to settle for 40 min. These cells were fixed to the wells by incubating with 100 μl of 0.025% glutaraldehyde (Kodak) in PBS at room temperature (RT)
for 20 min. The plates were washed three times with PBS and then filled with 200 μl/well of gelatin solution [200 μg/ml gelatin with 0.1% NaN₃ in PBS]. These cell-coated ELISA plates were stored at 4°C for up to three months.

In the assay, the plates were prewashed with PBS, and then hybridoma culture supernatants or culture medium were added to the wells (100 μl/well) for a two hour incubation at 37°C. The plates were washed four times with PBS and one time with 0.2% Tween-20 in PBS (PT). The second antibody, 50 μl of rabbit anti-mouse IgG [heavy and light chain specific (Cappel, Cooper Biomedical, Malvern, PA)] at a 1:100 dilution in assay buffer [10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1 mM MgCl₂, 10% IgG free fetal calf serum (GIBCO)] was then added and incubated for one hour at 37°C. The washing procedure was repeated, and 50 μl of protein A-β-galactosidase (Zymed, Burlingame, CA) were added, at a 1:200 dilution in assay buffer, to each well for another one hour incubation at 37°C. After the washing steps, cells in each well were incubated with 100 μl substrate, o-nitrophenyl-β-D-galactopyranoside (Sigma) [4 mg/ml in assay buffer with 0.7 μl/ml β-mercaptoethanol (Bio-Rad, Richmond, CA)] for 40-60 min. The reaction was stopped by adding 1 M Na₂CO₃ (100 μl/well). Color intensities were evaluated visually or measured by reading the absorbance at 410 nm (A₄₁₀).
MAb Ig Subclass Determination

The subclass of each MAb was determined by Ouchterlony double diffusion in agar using concentrated hybridoma supernatant and rabbit anti-mouse Ig heavy chain-specific antibodies (Litton Bionetics, Kensington, MI). The Ig subclasses were confirmed by ELISA using a mouse MAb subisotyping kit (Hyclone).

Indirect Immunofluorescence Assay

The assay was performed in U-bottomed Immunolon II microtiter plates. In each well, 1x10^6 swine PMNC (10 µl of 1x10^6 cells/ml in PBS) were incubated with 100 µl of concentrated supernatant at 4°C for one hour. The appropriate concentration of supernatant in this assay was first determined by titrating the concentrated supernatant against swine PMNC. The concentration chosen was on the plateau region of maximum binding. The plateau level concentration was then used, in triplicate, to test the percentage binding to swine PMNC of three different SLA types. For the negative control, culture medium was used. After the one hour incubation, the cells were washed four times with PBS containing 0.1% BSA and 0.1% NaN_3 (PBS/A/A). The second antibody, 50 µl of fluorescein conjugated goat anti-mouse Igs (IgA+IgG+IgM) (Cappel), at a 1:20 dilution in PBS, was added to each well for another one hour incubation at 4°C. The cells were then washed four times with PBS/A/A to remove the
unbound Ab and fixed by incubating with 70% cold ethanol for 10-15 min followed by a wash with PBS. The fixed cells were resuspended in PBS and analyzed on an EPICS 752 flow cytometer (Coulter, Hialeah, FL) with a microsampler delivery system. This flow cytometer is equipped with a five watt argon ion laser and was operated at a wavelength of 488 nm and power of 350 mW. Fluorescein fluorescence was detected after passage through 530 nm long-pass and 560 nm short-pass filters. Analysis of 2.5x10^4 cells, gated on forward angle light scatter, was performed on each tested sample. Percentages of fluorescent cells were calculated by using the EASY-88 "INTGRA" computer program (Coulter). The data were also calculated as histograms in which the cell frequency was plotted in relation to the logarithmic amplified fluorescent intensity.

**MAb Cytotoxic Activity**

Complement fixation ability of the MAb was determined by a direct complement-mediated cytotoxicity test which was performed in the U-bottomed Immunolon II microtiter plates. In each well, 6x10^4 swine SLA^d^ PMNC [10 µl of 6x10^6 cells/ml in RPMI-1640 with 10% heat-inactivated FCS] were mixed with 10 µl of serially diluted ascites or concentrated supernatants, and 10 µl of guinea pig serum (complement) at a 1:2 dilution, and incubated at 37°C for one hour. The Sp2/0 normal ascites and culture medium served as negative controls.
and the swine anti-SLA<sup>d</sup> alloantiserum served as a positive control. Following the one hour incubation, the cells were washed three times with PBS and stained with 10 µl per well of propidium iodide (PI) (0.5 mg/ml in PBS) for 10 min. The cells were then washed two times with PBS to remove the extra dye and resuspended in PBS. Cell viability was determined by PI dye exclusion. Propidium iodide fluorescence data were collected on 2x10<sup>4</sup> cells of each sample and analyzed by using the EPICS 752 flow cytometer, as described earlier, with the modification of using a 590 nm long-pass filter for collecting the PI fluorescence. Results were expressed as percentages of PI fluorescent cells, which represented the percentages of dead cells.

**Cell Surface Protein Labeling With ^125^I**

Lactoperoxidase-catalyzed iodination was used to label cell surface proteins. One milliliter of a swine suspension (5x10<sup>7</sup> cells/ml in PBS) was mixed with 15 µl of lactoperoxidase (Sigma) solution (167 U/ml in PBS), 1 mCi of carrier-free Na<sup>125</sup>I (Amersham, Arlington Heights, IL), and 15 µl of 0.03% H<sub>2</sub>O<sub>2</sub> (Sigma). After a 1.5 min incubation at RT, another 15 µl of lactoperoxidase and 15 µl of 0.03% H<sub>2</sub>O<sub>2</sub> were added for a further 1.5 min incubation. This last step was repeated one additional time. The reaction was then stopped by washing the cells with serum-free RPMI-1640 medium (GIBCO) four times. Iodinated cells were then solubilized at 1x10<sup>8</sup> cells per ml in
extraction buffer [0.5% Nonidet P-40 (NP-40) in 10 mM Tris, pH 7.2, 0.15 M NaCl, 0.02% NaN₃ containing 1 mM phenylmethyl-sulfonyl fluoride (PMSF), 100 µg/ml trypsin inhibitor, 1 µg/ml pepstatin and 10 µg/ml leupeptin] for 15-30 min on ice. After removal of the insoluble material by centrifugation, the cell extract was precleared by a treatment with Sp2/0 normal ascites for one hour at 4°C and with fixed Staphylococcus aureus Cowan I bacteria (SaCI) (Pansorbin cells, Calbiochem, La Jolla, CA) two times, one hour incubation at 4°C each, to remove material bound nonspecifically.

Immunoprecipitation and SDS-PAGE

The molecular weights of the antigens bound by the MAb were determined by immunoprecipitation of radiolabeled cell extracts, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Radiolabeled cell extracts (60 µl) were incubated with 20-40 µl of ascites fluid or 150-200 µl of concentrated supernatant, at 4°C overnight. The Sp2/0 normal ascites or Sp2/0 culture supernatant were used as negative controls, and swine anti-SLAd alloantiserum served as a positive control. The immune complexes were then precipitated by incubation with 200 µl SaCI (10% suspension in PBS) at 4°C for one hour and then washed four times as described (19). For those MAb that do not bind protein A directly (IgGl, IgM, IgA), precipitates were formed by using rabbit anti-mouse Ig heavy chain specific antibody (Litton
Bionetics) treated SaCI. After immunoprecipitation, materials bound to the MAb were dissociated by boiling 5 min in sample buffer containing 2% SDS and 5% β-mercaptoethanol. The SaCI was removed by centrifugation. Electrophoresis was performed on 12.5% polyacrylamide slab gels. Each gel with $^{125}$I samples was then stained with Coomassie Blue, dried, and exposed to XAR-5 X-ray film (Kodak) with an intensifying screen at -70°C for 2-10 days.

**Binding Activity Test of MAb 7-34-1 to Radiolabeled β$_2$m**

Binding activity of MAb 7-34-1 to radiolabeled β$_2$m was tested in the presence of a second antibody, anti-mouse Ig antibody. Purified swine β$_2$m was a gift from Dr. Joan Lunney, Animal Parasitology Institute, USDA, Beltsville, MD. Swine β$_2$m was iodinated using the lactoperoxidase enzymobead method (Bio-Rad). In the assay, aliquots of $^{125}$I-β$_2$m (5x10$^4$ cpm/aliquot) were first incubated in duplicate with serial dilutions (1:10, 1:100, and 1:1000) of either MAb 7-34-1 (ascites), MAb 74-11-10 (ascites), goat anti-swine β$_2$m antiserum, normal goat serum, or PBS containing 1% BSA (PBS/A), for 24 hours at 4°C. The goat anti-swine β$_2$m antiserum and normal goat serum were also gifts from Dr. Joan Lunney. One of four dilutions (1:2, 1:5, 1:20, 1:80) of the second antibodies, rabbit anti-mouse IgGs or rabbit anti-goat IgGs (Cappel), was added for a further 36 hr incubation at 4°C to form precipitates. After centrifugation, 200 μl of cleared
supernatants (sup) were collected for counting in a gamma counter (TM Analytic, Elk Grove Village, IL). The percentage binding activity of each sample to the \( \beta_2^m \) was calculated as:

\[
\frac{(\text{cpm of sup from PBS/A control} - \text{cpm of sup from Ab tested})}{(\text{cpm of sup from PBS/A control})} \times 100\%
\]

Cytotoxicity Inhibition Test of MAb 7-34-1 by Swine \( \beta_2^m \)

A two-stage NIH standard microcytotoxicity test was performed in Terasaki plates (Falcon, Oxnard, CA) as previously described (16) except that purified swine \( \beta_2^m \) was added at the first stage to detect possible inhibition of cytoxicity. In brief, \( 2 \times 10^3 \) swine \( \text{SLA}^d \) PMNC (1 \( \mu l \) of \( 2 \times 10^6 \) cells/ml in RPMI-1640 with 10% FCS) were incubated with 1 \( \mu l \) of serially diluted ascites from MAb 7-34-1 or MAb 74-11-10 and 2 \( \mu l \) of swine \( \beta_2^m \) at concentrations of 0 \( \mu g/ml \), 1 \( \mu g/ml \), 5 \( \mu g/ml \), 25 \( \mu g/ml \), and 125 \( \mu g/ml \), for 30 min at RT. Rabbit anti-swine xenoantiserum and normal swine serum were used at a 1:8 dilution as controls. After the first 30 min incubation, 5 \( \mu l \) of guinea pig complement at a 1:2 dilution were added to each well for a further 60 min incubation at RT. The percentage of dead cells in each well was determined microscopically.

Isolation of PMNC from Other Species

To study the possible interspecies crossreactivity of MAb 7-34-1, PMNC from several species were isolated for use in the
ELISA procedure. Briefly, PMNC were isolated from heparinized blood from human, sheep, cattle, goat, mouse, and rat by Ficoll-Hypaque (density 1.077, Sigma) centrifugation. Residual erythrocytes were lysed by treatment with Tris-$\text{NH}_4\text{Cl}$ as described (16).
RESULTS

Cell Fusion and ELISA Screening

Five fusion experiments were performed. As shown in Table
1, culture supernatants from 20 stable hybridoma lines were
identified which had reactivity against swine PMNC in an ELISA
screening procedure. Among these 20 MAb, eight of them were
derived from the fusion following the SLA$^c$ PMNC immunizations,
four MAb from the SLA$^a$ T cell immunizations, two MAb from the
SLA$^d$ T cell immunizations, one MAb from the SLA$^d$ activated
PMNC immunizations, and five MAb from the SLA$^a$ PMNC
immunizations.

MAb Subclass and Cytotoxic Activity

The Ig heavy chain subclasses of the 20 MAb produced are
summarized in Table 1. Isotypes of these MAb include nine
IgGl, seven IgG2a, three IgM, and one IgA. Four of the MAb,
7-34-1 (IgG2a), 8-34-4 (IgG2a), 10-14-1 (IgM), and 12-22-2
(IgM), showed direct cytotoxicity on swine PMNC, i.e., these
four MAb had the ability to fix complement (Table 1).

Indirect Immunofluorescence Assay

The percentage of swine PMNC, of three SLA haplotypes,
which bound the 20 MAb, was determined by indirect immuno­
fluorescence staining. The results are shown in Table 2.
Less than 5% of the cells were stained when the culture medium
was used as a negative control. The standard error of each
Table 2. Percentage of swine peripheral mononuclear cells reactive with MAb as determined by indirect immunofluorescence

<table>
<thead>
<tr>
<th>MAb</th>
<th>Mean Percentage of Fluorescent PMNC*</th>
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<tbody>
<tr>
<td></td>
<td>SLA^a</td>
</tr>
<tr>
<td>7-5-9</td>
<td>97</td>
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<tr>
<td>7-12-3</td>
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<tr>
<td>12-65-3</td>
<td>55</td>
</tr>
</tbody>
</table>

*Mean percentage of fluorescent PMNC was determined by the use of a flow cytometer, as described in the text.
triplicate sample was less than 2% of the mean (data not shown). The 20 MAb tested were found to have similar reactivity on swine PMNC of all three SLA haplotypes, \(a\), \(c\), and \(d\). Although cells from these three haplotypes were used as immunizing antigens for fusion experiments, none of the MAb produced were haplotype specific. Ten of the MAb stained more than 95% of swine PMNC and they were considered as possible candidates for anti-SLA class I specific MAb. The other 10 MAb recognized antigens on subpopulations of cells. Figure 1 shows fluorescent profiles of the 20 MAb on SLA\(^a\) PMNC, with the ordinates representing relative cell number and the abscissas representing relative logarithmic fluorescence intensity. Nine of the MAb, 7-5-9, 7-34-1, 7-59-7, 7-77-4, 8-34-4, 8-59-2, 12-27-3, 12-43-6, and 12-47-4, showed a similar staining pattern on the whole PMNC population. MAb 9-89-3 also stained the whole cell population but showed a different staining pattern. This may be a reflection of the unique isotype (IgA) of this MAb. MAb 7-12-3, 7-26-5, 7-40-6, and 8-8-6 stained 8-30% of the cells. MAb 7-212-2, 8-115-1, 9-65-1, 10-14-1, 12-22-2, and 12-65-3 stained 40-90% of the cells. Among this last group of MAb, 9-65-1 and 10-14-1 are unique because they showed bimodal staining of the PMNC.

**Immunoprecipitation of Cell Surface Antigens**

Immunoprecipitation experiments were performed with the MAb, by using \(^{125}\)I surface-labeled cell extracts, to determine
Figure 1. Binding of a panel of mouse anti-swine PMNC MAb to swine PMNC by an indirect immunofluorescence assay. Fresh culture medium was used as a negative control. The SLA haplotype of the swine cells tested was SLA^a. Relative cell number is plotted against relative fluorescence intensity.
Relative Fluorescence Intensity (log)
the molecular weights of the antigens detected by the MAb. The precipitated molecules were subjected to SDS-PAGE under reducing conditions, followed by autoradiography. Among the 20 MAb tested, six gave reproducible positive results, and the resulting autoradiograms are shown in Figure 2. As a positive control, the immunoprecipitation of a SLA^ cell extract with anti-SLA^ alloantiserum gave two bands, 50 kd and 12 kd (lane 3), corresponding to the two chains of the class I MHC molecule. No class II molecules are detectable on this autoradiogram (lane 3). The lack of detectable class II protein bands may be due to the low level of class II antigens on peripheral blood cells or to low anti-class II antibody in the antiserum. Sp2/0 normal ascites (lanes 1, 4, and 6) and Sp2/0 culture supernatant (lane 9) were used as negative controls. Among the MAb tested, MAb 7-34-1 gave the same banding pattern (lane 2) as the alloantiserum control (lane 3), i.e., two characteristic bands of class I antigens, a 50 kd band (heavy chain) and a 12 kd band (β_2m). This indicates that MAb 7-34-1 has most likely precipitated an SLA class I molecule. Immunoprecipitation experiments with the other 14 MAb failed to produce any bands on the autoradiograms. Some possible reasons that the other MAb tested did not produce bands in the immunoprecipitation may be that the nature of the antigen gave poor labeling with ^125_I, a small amount of antigen was expressed on the cells, and/or too few cells
Figure 2. Molecular weight determination of antigens precipitated by MAb. The 125I-labeled cell surface proteins, from peripheral mononuclear cells (SLA\(^d\)), were precipitated from the concentrated culture supernatants (sup) or ascites fluids by MAb and S. aureus Cowan I. The precipitates were analyzed on 12.5% acrylamide gels under reducing conditions. MAb used to give the autoradiograms are Lane (2) 7-34-1 (ascites); Lane (5) 10-14-1 (ascites); Lane (7) and Lane (8) 12-65-3 (ascites) [Lane (7), long exposure; Lane (8), short exposure]; Lane (10) 7-12-3 (sup); Lane (11) 7-26-5 (sup); and Lane (12) 8-8-6 (sup). Negative controls of Sp2/0 ascites are shown in Lanes (1), (4), and (6) and of the Sp2/0 supernatant in Lane (9). The positive control (anti-SLA\(^d\) alloantiserum) is in Lane (3).
expressed the antigen. Table 3 summarizes the molecular weights of the precipitated cell surface proteins from the six MAb. Only MAb 7-34-1 (the putative anti-SLA class I antibody) was chosen for future study.

**Test for binding of MAb 7-34-1 to \( \beta_2^m \)**

Monoclonal antibody 7-34-1 precipitated antigens with the same molecular weights as the MHC class I heavy chain and \( \beta_2^m \). This fact, coupled with the flow cytometer profile of the antibody on PMNC, strongly suggests that it is an anti-class I MAb. The antigenic epitope of this MAb might be on the SLA class I heavy chain, on \( \beta_2^m \), or on the class I heavy chain complexed with \( \beta_2^m \). To further characterize whether the antigenic determinant of MAb 7-34-1 was on \( \beta_2^m \), experiments to detect the possible binding of MAb 7-34-1 to radiolabeled swine \( \beta_2^m \) and experiments to measure inhibition of cytotoxicity of MAb 7-34-1 in the presence of free \( \beta_2^m \) were performed. In the \( \beta_2^m \) binding test, both MAb 7-34-1 and MAb 74-11-10 were tested and appropriate anti-Ig Ab was added as a second Ab to form precipitates. Results (see Fig. 3) showed that neither MAb 7-34-1 nor MAb 74-11-10 showed binding to the radiolabeled \( \beta_2^m \) at all the dilutions used and that the goat anti-mouse \( \beta_2^m \) positive control gave a maximum binding of 45% at a 1:10 dilution of anti-\( \beta_2^m \) antiserum and a 1:2 dilution of the second antibody. In the cytotoxicity inhibition assay, the complement fixation ability of MAb 7-34-1 (IgG2a) and MAb
Table 3. Summary of molecular weights of proteins immunoprecipitated by monoclonal antibodies

<table>
<thead>
<tr>
<th>MAb</th>
<th>Molecular Weights ($\times 10^3$) of Immunoprecipitated Proteins Shown in Figure 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-12-2</td>
<td>90, 60, 49 (Lane 10)</td>
</tr>
<tr>
<td>7-26-5</td>
<td>90, 60, 52 (Lane 11)</td>
</tr>
<tr>
<td>7-34-1</td>
<td>50, 12 (Lane 2)</td>
</tr>
<tr>
<td>8-8-6</td>
<td>160, 125 (Lane 12)</td>
</tr>
<tr>
<td>10-14-1</td>
<td>23 (Lane 5)</td>
</tr>
<tr>
<td>12-65-3</td>
<td>200, 180, 30 (Lanes 7 and 8)</td>
</tr>
</tbody>
</table>
Figure 3. Test for binding of MAb 7-34-1 to radiolabeled swine $\beta_2$m. MAb 7-34-1 (ascites) (•••••), MAb 74-11-10 (ascites) (o--o), goat anti-$\beta_2$m serum (Δ--Δ), and normal goat serum (□--□) were used at three dilutions, 1:10, 1:100, and 1:1000. The second antibodies, anti-mouse IgG or anti-goat IgG, were used at 1:10 dilutions.
The image shows a graph that represents the binding activity of $\beta_2$m as a function of antibody dilution. The graph has a logarithmic scale for antibody dilution on the x-axis and binding activity on the y-axis. There are multiple lines on the graph, each representing a different condition or group. The graph suggests a decrease in binding activity with increasing antibody dilution.
74-11-10 (IgG2b) was not inhibited when the soluble β₂m was added. These results provide evidence that the epitope recognized by MAb 7-34-1 is not on β₂m but more likely on the class I heavy chain.

Comparison of MAb 7-34-1 and MAb 74-11-10 Binding to PMNC

A comparison was made of the antigenic specificity of MAb 7-34-1 with MAb 74-11-10, developed by Pescovitz et al. (13). The Ab titration curves of protein-A purified MAb 7-34-1 and MAb 74-11-10 on PMNC of three SLA types were analyzed, in duplicate, by indirect immunofluorescence staining followed by flow cytometric analysis. The binding activities of these MAb on swine PMNC are presented as the percentages of fluorescent cells. As shown in Figure 4, MAb 7-34-1 gave similar titers on all three types of cells, whereas MAb 74-11-10 showed strong binding reactivity to SLA^a and SLA^d cells but not to SLA^c cells. Monoclonal antibody 74-11-10 was positive to SLA^c cells only at the highest Ab concentration (1 mg/ml), and an approximately 100-fold more concentrated MAb was required to cause the same level of binding of the SLA^c cells as to the cells of the other two haplotypes. These data indicate that MAb 7-34-1 and MAb 74-11-10 recognize different epitopes on SLA class I molecules.
Figure 4. The titration curves of MAb 7-34-1 and MAb 74-11-10 on swine peripheral blood mononuclear cells by an indirect immunofluorescence assay. Protein-A purified MAb 7-34-1 (••••) and MAb 74-11-10 (o---o) were titered, in duplicate, on swine cells from three SLA inbred lines: (A) SLA^a, (B) SLA^c, and (C) SLA^d. Percentages of positive cells were determined by flow cytometric analysis.
A

SLA\(^{a}\) CELLS

Fluorescent Cells (\text{%})

Antibody Concentration (mg/ml)
Figure 4. Continued
B

SLA\(^{+}\) CELLS

Fluorescent Cells (%)

Antibody Concentration (mg/ml)
Figure 4. Continued
SLA\(^d\) CELLS

Fluorescent Cells (%)

Antibody Concentration (mg/ml)

100
80
60
40
20
0

1
10\(^{-1}\)
10\(^{-2}\)
10\(^{-3}\)
10\(^{-4}\)
10\(^{-5}\)
10\(^{-6}\)
Finally, interspecies cross-reactivity of MAb 7-34-1 was analyzed by an ELISA on glutaraldehyde fixed PMNC from human, sheep, cattle, goat, and mouse. Results (data not shown) were that MAb 7-34-1 did not cross-react with the PMNC isolated from any of these species. However, MAb 7-34-1 did react with PMNC from a variety of breeds of commercial pigs. Thus, MAb 7-34-1 seems to be specific for swine antigens.
DISCUSSION

In this report, we describe the production and characterization of a panel of 20 murine MAb with specificity for swine PMNC. In our study, swine PMNC, T cells or PHA activated PMNC from particular SLA inbred miniature swine were used as immunogens for xenogeneic immunizations. No SLA haplotype specific MAb were obtained. The MAb of special interest that was obtained is 7-34-1. Based on immunoprecipitation studies, flow cytometric profiles, and competition studies with radiolabeled \( \beta_2 \text{m} \), it seems highly likely that this MAb is specific for the heavy chain of SLA class I antigens.

Two other laboratories have also reported the development of murine MAb to SLA class I antigens by fusions after xenogeneic immunization (13, 14). So far, two class I specific MAb, 74-11-10 and PT85 have been obtained [reviewed in (4)]. The immunogens used to develop 74-11-10 and PT85 were thymocytes (SLA\(^d\)) from SLA inbred miniature swine (13) and thymocytes from domestic swine (14), respectively.

A comparison of the epitope specificity of our MAb, 7-34-1, with 74-11-10 and PT85 is interesting. The different binding activities of 7-34-1 and 74-11-10 on swine PMNC of SLA inbred lines suggest that these two MAb recognize different determinants on SLA class I molecules. Monoclonal antibodies 7-34-1 and PT85 probably also recognize different epitopes on
class I molecules because PT85 has been shown to cross-react with an antigenic determinant on goat cells (14), whereas our MAb, 7-34-1, did not cross-react with PMNC isolated from the goat.

Besides fusions after xenogeneic immunization, another approach available to develop MAb as SLA typing reagents is to test pre-established MHC specific MAb from other species on swine cells for cross-reactivity. For this alternative approach, several anti-mouse or anti-human class II monoclonal antibodies have been reported with cross-reactivity to swine class II antigens (8, 20). However, most mouse anti-human HLA class I specific MAb do not cross-react with SLA class I antigens (13, 21). Therefore, it will be necessary to perform further fusions to prepare MAb for use as SLA typing reagents. Changes could be made in the types of cells used for immunization, the immunization protocol, and the types of mice immunized to increase the possibility of producing haplotype-specific reagents. For instance, alloimmunization of mice with mouse L cells transformed with SLA class I genes (9) or with mouse cells isolated from transgenic mice expressing SLA class I genes (22) might result in a high immune response to particular SLA antigens. In the human system, it has been reported the immunizing mice with intact human cells results in the production of non-haploype specific monoclonal antibodies such as anti-β2m, anti-class I, and anti-class II,
whereas immunizing mice with purified papain solubilized MHC glycoproteins as immunogens substantially increase the chance of obtaining MAb to haplotype specific determinants (23). Perhaps a similar procedure could be undertaken in the swine system.

In conclusion, we have produced 20 MAb to cell surface antigens on swine PMNC. These MAb may be useful for the study of the immune system in swine. One of the MAb produced, 7-34-1, recognizes class I antigens of SLA haplotypes a, c, and d in an equivalent manner. This MAb should be especially useful as a general anti-SLA class I reagent for experiments on NIH miniature swine.
REFERENCES


SUMMARY AND DISCUSSION

In order to understand the genetic and molecular structure of the SLA complex, this research has addressed three important issues: 1. The linkage of the swine Glo enzyme locus and the SLA complex; 2. The mapping of the swine class III genes, C2, Bf and C4; 3. The development of anti-SLA MAbs. In these investigations, NIH miniature swine of known SLA haplotypes were used as the experimental animals. Modern biotechnical procedures, such as monoclonal antibodies and recombinant DNA techniques, were employed. Data in this dissertation included the first demonstration of the linkage of GLO to SLA and the first report of the mapping of the swine C2 and Bf genes to the SLA complex. Furthermore, the successful production of a general anti-SLA class I MAb was described.

The study of swine GLO polymorphism was initiated because a conserved linkage between Glo and the MHC has been demonstrated in humans, mice and rats. Section I of this dissertation, the linkage of Glo to the SLA complex was suggested on the basis of quantitative differences in GLO levels among SLA haplotypes. Although the usefulness of this linkage as a genetic marker for the SLA complex may be limited because of the low differences among haplotypes, the demonstration of this evolutionary conservation in swine may be significant.
The class I and class II molecules of swine have been identified and characterized. The lack of information on swine class III proteins had delayed the study of the SLA class III region until recent advances in DNA technology allowed more direct examination. Section II of this dissertation, a close linkage of the swine C2 and Bf genes and the linkage of the C2-Bf region to the SLA class II genes were clearly demonstrated by detection of the DNA RFLPs in miniature swine using heterologous human C2 and Bf cDNA probes. Section III of this dissertation, a close association of C4 and class II genes was shown by using a similar approach. These data demonstrated the evolutionary conservation of class III genes in the swine genome. The SLA complex can now be tentatively characterized as DQDR[(C2-Bf)-(C4)]BA. Moreover, the TaqI and MspI (Appendix, Figure 1) DNA polymorphisms in the C2-Bf region and the BamHI, PvuII, and SstI polymorphisms in the C4 region may also be useful as genetic markers of the SLA complex.

Data presented in Sections II and III of this dissertation could lead to more complete characterization of the SLA complex. Further studies on the class III region of swine, utilizing the same approach, could include:

(1) Analysis of the Bf and C4 RFLPs in miniature swine of recombinant haplotype $f$. Haplotypes $g$ and $f$ are the two identified, independent intra-SLA recombinants in NIH
Both recombinants express the class I genes of haplotype g and the class II genes of haplotype d. The recombinant g was used in the earlier studies in Sections II and III to show that the class III DNA RFLPs segregated with the class II genes. It would be interesting to analyze the Bf and C4 RFLP patterns in the recombinant f. If the RFLP patterns in haplotype f are identical to the patterns of haplotype g, it would suggest that both independent recombinants have the crossover site(s) located between the SLA class I and the class II-class III regions. On the other hand, if the recombinant f produces identical class III DNA RFLP patterns to haplotype g, but different from those of recombinant g, then the data would imply that haplotypes g and f have different crossover sites and the swine class III genes could be mapped between the class I and the class II regions.

(2) Detection of possible C2 polymorphisms in miniature swine. The search for possible swine C2 RFLPs could be continued by using different probes and/or different restriction endonucleases, as described in the Section II.

(3) Detection of additional DNA polymorphisms of swine 21-OHase gene(s) and the possible association of 21-OHase and C4 in miniature swine. A close linkage of C4 and 21-OHase genes has been found in humans and mice. It is very possible that a close linkage would also be detected in swine. Inasmuch as there is very high homology between bovine
21-OHase and swine 21-OHase at the protein level, the detection of additional RFLP patterns of swine 21-OHase gene(s) might be accomplished with a bovine 21-OHase cDNA probe. The demonstration of C4 and 21-OHase linkage may still be accomplished by detection of overlapping restriction fragments using both 21-OHase and C4 probes.

(4) Determination of the number of C4 genes in miniature swine. There are two copies of both C4 and 21 OHase genes in the class III regions of humans and mice. The 21-OHase genes have been mapped adjacent to both C4 genes. If the swine 21-OHase could be placed adjacent to the swine C4, it would then be possible to develop proper heterologous probes specific to the 5' end and 3' end of both C4 and 21-OHase genes to determine the number of C4 genes in miniature swine. If certain common bands could be detected using probes specific to the 3' end of C4 and the 5' end of 21-OHase and other overlapping restriction fragments could be detected using probes specific to the 3' end of 21-OHase and the 5' end of C4, the existence of more than one copy of C4 genes in the swine class III region would be suggested.

(5) Determination of the relative orientation of and distance between swine C2, Bf, and C4 genes. The relative orientation of and distance between class III genes in miniature swine could be determined by detecting overlapping restriction fragments using probes specific to the 5' and/or
the 3' ends of each class III gene. Because the distance between human Bf and C4A genes is about 30 kd, detection of overlapping restriction fragments between swine Bf and C4 may require the use of different restriction enzymes and field inversion gel electrophoresis (26,88) for generation of larger restriction fragments.

(6) Determination of the orientation of swine class III genes with respect to the class I and class II genes. The order of the mouse class III genes from the centromeric to the telomeric end of the mouse chromasome has been determined to be 21-OHaseB, C4, 21-OHaseA, Slp, Bf, C2. However, the orientation of class III genes with respect to the class I and class II loci in humans and swine is still unknown. Determination of the exact order of the genes in the SLA complex could be accomplished by using class I, class II, and class III probes and pulse-field gel electrophoresis (24) which could establish long-range genomic restriction maps.

It would also be interesting to determine whether or not additional genes, e.g., TNF-α and TNF-β, fall into the SLA complex. All of these experiments could contribute to a better understanding of the organization of the SLA complex and show the extent of general conservation of the MHC complex in vertebrates.

A molecular map of the SLA complex could also be established by detecting overlapping clones and chromosomal
walking. One of the goals for the SLA researchers has been to identify, isolate and characterize the genes of the SLA complex. Construction of swine genomic and/or cDNA libraries has been initiated. However, only class I genes have been isolated so far. The molecular cloning and characterization of the SLA genes could be very important in future studies of the structure and functional relationship of a specific SLA gene and its gene product.

The application of hybridoma technology to the study of the MHC has also contributed significantly to the understanding of the structure and function of this important gene complex. Section IV of this dissertation, production and characterization of a panel of 20 murine MAb to swine peripheral blood cell surface antigens, including one MAb to SLA class I antigens, was described. Future experiments involving the use of various types of immunogens and/or immunization protocols for improvement of the production of MAb as typing reagents were also described in the Section IV. To continue this project, it would also be interesting to determine if the MAb 10-14-1, described in Section IV, is SLA class II specific. This MAb reacted primarily with B cells, macrophages and subsets of T cells (Joan Lunney, personal communication, US Department of Agriculture, Beltsville, Maryland) and precipitated a 23 kd protein which may be the β chain of the class II molecule. Further experiments, e.g.,
functional assays and/or immunoprecipitation of $^{35}\text{S}$-labeled cells extracts may help to further characterize the antigen specificity of this MAb.

In conclusion, using SLA inbred and recombinant miniature swine as animal models, this dissertation reports data on mapping of class III complement genes and the Glo enzyme locus to the SLA complex, and on producing MAb to swine lymphocyte antigens. Swine are important both in biomedical research and for their economical value. An understanding of the organization of the SLA complex and SLA genes and their gene products is essential because of the utmost important role of the MHC in biological phenomena. It is believed that a complete characterization of the SLA complex will be accomplished with specific restriction fragment or nucleotide probes and MAb to identify specific SLA genes and their products.
LITERATURE CITED


Figure 1. Southern blot analysis of MspI digested miniature swine genomic DNA by using the 0.66 kb human Ba probe. The miniature swine used are as indicated: (A) miniature swine of SLA haplotypes a, c, d, and g; (B) a family (SLA a/d sire, SLA a/d dam and three offspring: two SLA d/d and one SLA a/d). SLA haplotype corresponding to the DNA sample is indicated under each track. The sizes of restriction fragments are estimated on the basis of HindIII digested lambda-phage DNA fragments.
A

Msp I

1 2 3 4

(kb)

B

Msp I

1 2 3 4 5

(kb)

SLA haplotype

a c d g

a/d a/d d/d d/d a/d

(♂) (♀)
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