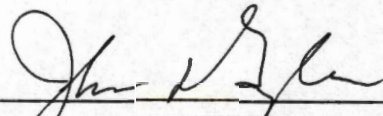


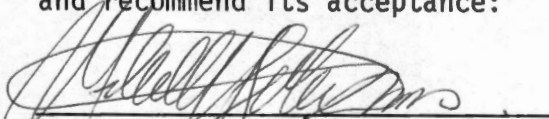
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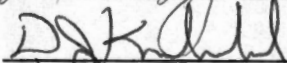
I am submitting herewith a thesis written by Joan E. Bray entitled "The Swine Leukocyte Antigen System and Its Relationship to Vascular Allograft Survival." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

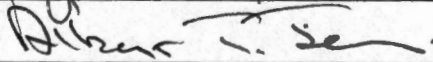


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THE SWINE LEUKOCYTE ANTIGEN SYSTEM AND ITS RELATIONSHIP
TO VASCULAR ALLOGRAFT SURVIVAL

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Joan E. Bray
March 1988

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Thesis

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DEDICATION

This thesis is dedicated to Mitchell H. Goldman, M.D., who bestowed upon me the stone of ambition.



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ABSTRACT

Miniature swine which have been inbred and defined for their A-0 and MHC antigens (SLA) offer the opportunity to apply immunogenetic analyses of vascular allografts in a large animal model. Accordingly, internal jugular veins were transplanted as interposition grafts to the carotid arteries of 14 unmodified SLA-typed miniature swine. Transplants were performed between size- and age-matched reciprocal pairs sharing zero, one or two haplotypes. Prior to transplantation, pairs were lymphocyte crossmatched (XM) for possible SLA antibody (Ab) and only pairs with a negative XM were used. One week post-transplant and weekly thereafter, recipient sera were XM against donor lymphocytes. Allograft patency (survival) was monitored with a hand-held Doppler immediately post-transplant and then weekly until graft rejection (closure). Two recipients were lost due to post-surgical complications. In MHC matched recipients, only one out of five developed a positive crossmatch (at three weeks). Nevertheless, its graft remained open at 16 weeks and one other recipient's graft remained open at nine weeks. The other MHC matched grafts were rejected at four, seven, and nine weeks (group mean ≥ 9.0 weeks). In the one-haplotype matched group, all recipients developed a positive XM (within six weeks) and all grafts were rejected by week seven (group mean = five weeks). In the two-haplotype mismatched group, three of four recipients developed a positive crossmatch (within three weeks) and all grafts were rejected by week four (group mean = 3.5 weeks). Notably, graft rejection invariably occurred coincident with or within two weeks following anti-donor antibody development in MHC mismatched recipients; whereas, in MHC matched

recipients, antibody development was rare and showed no connection with graft rejection. Analysis of the data demonstrated the following significant associations: MHC mismatched grafts were rejected an average of 4.3 weeks earlier than matched grafts ($p < .02$; t-test) and six of seven MHC mismatched recipients developed anti-donor antibody as compared to one of five matched recipients ($p = .044$; Fisher's exact test). Strong associations were shown when correlating SLA matching and T-cell sensitization. In the completely matched group 50% of the recipients gave a weak MLC response pre-transplant; the mean relative response being 3.5%, which was below the cutoff of 15% for determining positive versus negative results. Post-transplant, this group gave a mean MLR of 21.4%. The 1-haplotype matched group gave a mean relative response of 32.5% pre-transplant and 79.5% post-transplant. All recipients in the completely mismatched group had a positive MLC pre-transplant with a mean of 41.25% which rose to 79% post-transplant. In the completely matched group, 40% of the grafts remained open and had a negative post-transplant MLC. Of the 60% with closed grafts, one-third developed a positive MLC. In the 1-haplotype matched group, all recipients lost their grafts, 50% had a positive pre-transplant MLC, and all had a positive post-transplant MLC. The completely mismatched group had 100% graft closure, and 100% of the recipients gave a positive MLR. These results show there is not a direct correlation between cellular stimulation and graft rejection, although the degree of SLA matching and T-cell response in the MLR have a strong correlation.

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CHAPTER 1

INTRODUCTION

The area of vascular transplantation is a relatively new field when compared to other types of transplants. Because of the many types of antigen systems involved in the vasculature, including class I and II of the major histocompatibility complex, as well as specific vascular endothelial cell antigens, work has not progressed as rapidly in this system as it has in others, such as renal and cardiac transplantation. The vessels are harder to isolate cells from for identification of specific antigens, even though the major histocompatibility complex (MHC) is known to be present along with other antigens systems (1,2). There is an interest in this type of transplant for patients requiring by-pass surgery who do not have an adequate amount of veins which may be used as autografts (1,2). Some work has been done in the human, but without success; the allografts having been rejected (1,2). An animal model is needed to work out the problems of vascular transplantation.

Animals have been used as models for vascular transplantation for many years. Included among those used are dogs, mice, rats and miniature swine (mini-pigs). Due to limitations of size, the mouse is a poor model for this type of experimentation, even though genetically it is well-defined. The rat has been used quite successfully for this purpose (3-5), but anatomically is very dissimilar to humans. The same problem is encountered with the dog, even though genetically-characterized dogs have been used for vascular transplantation (1).

The mini-pig has been characterized for its MHC antigens and red blood cell groups (6,7). The reproductive capabilities make breeding quite simple, being able to produce genetically-characterized offspring in several generations. Last of all, the mini-pig is more anatomically similar to humans than dogs, making them an excellent clinical model for vascular transplantation (6). Due to these advantages over other animal models, the mini-pig was chosen as the model in which to perform and study vascular transplants.

The MHC [swine leukocyte antigen system (SLA)] of mini-pigs has been extensively studied. As with the human leukocyte antigen (HLA) system of man and the H-2 system of the mouse, the mini-pig has multiple class I and class II histocompatibility antigens. The class I antigens are glycoproteins with an SLA-encoded heavy chain of 42,000 daltons associated with β -2 microglobulin (11,000 daltons). Class II antigens have a light chain of 25,000 daltons and a heavy chain of 31,000 daltons, as determined by sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (8,9). Both chains are encoded by SLA genes. The exact locations of the class I and II SLA loci are unknown, but are expected to be similar to the human. Figure 1* shows the proposed locations of the various loci of this system (10).

Tissue distribution of MHC antigens has been reported to include the following: red blood cells were negative, all other tissues studied contained class I antigens. Certain tissues were class I positive and class II negative: platelets, cultured fibroblasts, smooth muscle cells,

*All figures and tables may be found in the Appendices.

aortic endothelium, and liver parenchyma (9). According to other sources, endothelial cells from kidney, spleen, and aorta are class II positive (10) as well as B lymphocytes, monocytes, macrophages and certain subpopulations of T lymphocytes. Rat endothelium obtained from the vena cava has been shown to be class I positive (4). SLA antigens have been shown to readily induce antibody production and kidney graft rejection (6,11). The antibody response to SLA is usually, although not exclusively, directed toward class I differences. The SLA antibodies are detectable by complement-dependent lymphocytotoxicity assays (6,12,13). SLA class II antigens are responsible for stimulation in mixed lymphocyte cultures (MLC) (6). The MLC is an assay based on cells from two different individuals being co-cultured, and responding by blastogenesis and cellular proliferation, i.e., a mixed lymphocyte response (MLR). This response reflects the ability of T lymphocytes (responder) of one individual to recognize different class II MHC antigens on the cell surface of the other individual (stimulator) (14). The extent of blastogenesis is determined by measuring uptake of tritiated thymidine (^3H TdR). A one-way MLC is a form of this assay when only one cell can respond, the stimulator having been treated with either mitomycin C or irradiation to inhibit blastogenesis.

Due to the immune activation of the SLA system, incompatibility at SLA has been closely linked to transplant rejection. Previous studies have been performed using skin-grafts between various SLA haplotypes (6,7,15) and extensive studies have been done in the area of renal transplantation (14,16,17). These studies found that MHC-matching had a marked prolonging effect on graft survival, especially class II

matching. The immunologic aspects of our vascular transplants have been studied in detail and are the subject of this thesis.

Sequential serologic and cellular assays (MLC) were undertaken in a herd of partially inbred miniature swine in an attempt to determine if a correlation exists between the degree of sharing of SLA antigens between donor and recipient, and the induction of anti-donor immune responses following vascular transplants. The results of these studies were then compared with corresponding Doppler studies of vascular patency as a measure of allograft survival (18).

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CHAPTER 2

MATERIALS AND METHODS

A. MATERIALS

Animals. The inbred mini-pigs used in this study were a gift of Dr. Robert Kirkman, Department of Surgery, Harvard Medical School, Brigham and Women's Hospital, Boston, MA. The inbred lines originated at the National Institutes of Health (NIH) under the direction of Dr. David H. Sachs, who did selective cross-breeding to produce SLA-defined offspring for transplant and immunogenetics studies (6). Individual mini-pigs were originally SLA-typed (for haplotypes) at the NIH and this was repeated upon receipt at the University of Tennessee (UT) to verify the typings. The individual class I and II SLA antigens have not been identified in this herd, but rather, the mini-pigs have been bred for particular SLA haplotypes, i.e., combinations of class I and II SLA products which are identical in pigs sharing the same haplotype.

The herd members all have red cell blood group type "O" (personal communication, Dr. Robert Kirkman). Swine red blood cells (RBC) have either type "A" or type "O"; no "B" type has been found (19). In the human, type "A" is agglutinated by anti(α)- A, type B by α B, and AB by both α A and α B. In swine, "A" cells are agglutinated by α A, "O" are not agglutinated by either α A or α B.

The SLA haplotypes of the mini-pigs used in this study were homozygous A, C, D or heterozygous combinations of these. The "B" haplotype is not present in any members of this particular herd, having been lost in the first generation (6).

The mini-pig herd was kept at the University of Tennessee College of Veterinary Medicine (UTCVM) Research Farm in special swine containment buildings. These buildings are temperature-controlled, and contain two rooms with adjustable pens for the pigs. The mini-pigs should be kept in a semi-controlled environment with temperatures ranging from 50°F to 80°F (personal communication, Dr. Fred Hopkins).

Certain pigs from this herd have been selected as breeders to produce more homozygous offspring and heterozygous offspring of specific types. The breeding phase of the overall pig study will be undertaken in the spring of 1988. An unplanned breeding took place during the spring of 1987, resulting in 28 offspring. The sows and all piglets were SLA typed to determine parentage and haplotypes for use in future studies.

Supplies/Reagents. RPMI-1640 tissue culture medium was used for all phases of this project and was purchased from American Scientific Products, Stone Mountain, GA. All disposable plasticware used (centrifuge tubes, tissue culture trays, etc.), and Hamilton syringes (50 μ l, 250 μ l, and 500 μ l) were also purchased from American Scientific Products, unless otherwise specified. Microtiter trays (96-well) for plating the MLC were Corning Cell-Wells, also purchased from American Scientific Products.

Fetal calf serum (FCS) was purchased from MA Bioproducts, Walkersville, MD.

Hepes, dimethylsulfoxide (DMSO), and Trypan blue as a powder were purchased from Sigma Chemical Co., St. Louis, MO. A 1% solution of Trypan blue was made in 2% disodium ethylenediaminetetraacetic acid

(EDTA). Mitomycin C purchased from Sigma Chemical Co. was used to inactivate the stimulator cells.

The Ficoll-Hypaque used was LSM, purchased from Organon-Teknica Corp., Durham, NC.

Rabbit complement was purchased from Grand Island Biological Corporation (GIBCO), Grand Island, NY.

All microlymphocytotoxicity assays were read using a phase-contrast microscope (American Optical).

The 72-well microtiter trays used for cross-matching and SLA typing were purchased from Robbins Scientific Corporation, Mountain View, CA.

EDTA was purchased from Fisher Scientific, Marietta, GA.

Barbitol buffer was purchased from Oxoid Ltd., England.

Media additives included gentamycin (from Elkins-Sihn, Inc., NJ), pyruvate, Pen-Strep, L-glutamine and non-essential amino acids (MA Bio-products).

The tritiated thymidine (20 μ Ci/ml) added to the MLCs was purchased from ICN Radiochemicals. A Skatron harvester was used to harvest the MLCs. Tritiated thymidine uptake was determined in a Beckman Model LS-330 liquid scintillation counter. Liquid scintillation fluid was Budget-Solve, purchased from Research Products International.

B. METHODS

Venipuncture. Blood samples for the immunological assays performed on the mini-pigs were obtained by one of two ways. The first was venipuncture of the precava, as suggested by the Agricultural Extension Service of UT (20), using sterile, 18 gauge, 6-inch needles attached to

either heparinized or plain 20 cc syringes. The second was external jugular venipuncture using a 21 gauge, 2-inch needle attached to plain 20 cc syringes. The contents of the latter syringes were immediately transferred to either red- (plain) or yellow-topped (ACD-treated) Vacutainer tubes using 18 gauge needles to avoid damaging the red blood cells. On a few of the mini-pigs with large, accessible ear veins, the blood was drawn from the ear while they were sedated with ketamine and acepromazine.

Serum Isolation. The red tops were centrifuged at 400 g at 4°C after the blood had had sufficient time to clot. The serum was transferred to Beckman microtiter tubes, which were appropriately labeled, and kept frozen at -80°C until needed.

Lymphocyte Preparation. The lymphocytes for the SLA, MLC and crossmatch assays were isolated from either heparinized or ACD-treated blood by Ficoll-Hypaque (FH) density centrifugation. The whole blood was diluted in half with RPMI-1640 tissue culture medium. (All RPMI-1640 used in any form in these studies contained L-glutamine (200 mM), Hepes (15 mM) buffer, and gentamycin (40 µg/ml).) Ten ml of the diluted blood were carefully layered over four ml of FH in 15 ml round-bottom, polystyrene tubes. The tubes were centrifuged at 365 x g for 30 min at room temperature (~ 22°C). The lymphocytes were removed from the FH interface using siliconized Pasteur pipettes and transferred to another set of sterile 15 ml centrifuge tubes. The tubes were filled with RPMI-1640 and centrifuged at 160 x g to pellet the lymphocytes and, at the same time, remove platelets and residual FH. The supernatant was decanted and the wash procedure repeated. After the second wash, the

cells were resuspended in RPMI-1640 with 20% FCS (RPMI-20%) to prepare for cryopreservation.

Cryopreservation. The isolated lymphocytes were suspended in RPMI-20% and viable cell counts determined by trypan blue dye exclusion (21). Viability was routinely greater than 90%. The cell suspensions were then adjusted to 10×10^6 /ml of viable cells and placed in the refrigerator for approximately 10 min to chill. Cryotubes (Sarstedt) were labeled with the appropriate freeze number and placed in a -20°C freezer for approximately 10 min to chill. When ready for freezing, an equal volume of 15% DMSO in RPMI-1640 was added dropwise to the cell suspension as the suspension was gently swirled. The cells were then aliquoted into the cryotubes, 1 ml per tube, and the tubes placed at the bottom of a -80°C chest freezer (Revco Ultra-Low). After 1.5 hr, the tubes were transferred to a liquid nitrogen refrigerator (Cryomed), where they were placed in boxes in the vapor phase of the liquid nitrogen for long-term storage.

Cell Preparation for Testing. The frozen cells were quickly thawed by gentle agitation of the cryotubes in a 37°C water bath. As soon as the last ice crystal had thawed, the contents were transferred to 15 ml centrifuge tubes. Ten ml of RPMI with 10% FCS (RPMI-10%) were added dropwise with mixing to the tubes. The tubes were then centrifuged at $160 \times g$ for 10 min to remove the DMSO. The assay for which the cells were intended determined whether or not they were to be kept sterile and which medium the cells were resuspended in:

MLC: sterile technique, RPMI-20% - 6 ml

SLA: unsterile technique, plain RPMI-1640 - 1 ml

XM: unsterile technique, plain RPMI-1640 - 1 ml

The cells for the MLC were adjusted to $2.0 \times 10^6/\text{ml}$. The cells for SLA type and crossmatches were adjusted to $2.5 \times 10^6/\text{ml}$. Viabilities were all greater than 90%, as tested by trypan blue dye exclusion.

Histocompatibility Testing. SLA typing was performed using the antiserum developed by Dr. Sachs (a gift from Dr. Kirkman) by immunizing the mini-pigs with SLA-incompatible skin grafts, and booster injections of lymphocytes (6). Antibody titers in the reagent sera were determined by repeating the SLA typing on previously typed mini-pigs using the Amos-Modified (Amos-Mod) version of the microlymphocytotoxicity technique used for human HLA testing (22) and scored by the suggested NIH technique (23). Two μl of mineral oil were dispensed into the wells of the microtiter trays to prevent the sera from evaporating when the trays were stored in a freezer. One μl of serum was added to each of the appropriate wells using a 50 μl Hamilton syringe. One μl of lymphocytes (adjusted to $2.5 \times 10^6/\text{ml}$) was then added to each well, making sure the cells and sera mixed. The lids were replaced and the tray incubated at 22°C for 30 min. A wash step was then performed, adding 10 μl RPMI-1640 with a 500 μl Hamilton syringe. The trays were allowed to set for 10 min, then flicked to remove residual sera, media, and oil. Five μl of rabbit complement were then added to each well using a 250 μl Hamilton syringe and the lids replaced. The trays were incubated at room temperature for 60 min. At the end of this period, the trays were flicked to remove excess complement, and a mixture of 2% EDTA/Trypan blue was added to inhibit further cell lysis and stain the dead cells. Reaction scores were assigned as follows: A "1" = 0-10% cell lysis; a "2" = 10-20% cell

lysis; a "4" = 20-50% cell lysis; a "6" = 50-90% cell lysis, and an "8" = 90-100% cell lysis. Cell lysis is indicative of complement-dependent antibody being present in the antisera which reacts specifically to the cell surface antigens.

SLA-typing sera were tested neat to 1:4096 using serial dilutions in RPMI with 10% FCS. Antisera #2331 had a specificity of α A and reached an end point of 1:1024 for complete cell lysis. Antisera #1921 was specific for α C, and reached an end point of 1:1024. Antisera #1919 had a specificity of α D and an endpoint of 1:512. A single lot of non-cytotoxic FCS was used throughout as a negative control to assess "background" cytotoxicity. Antisera α P23,30 was used at a dilution of 1:200 as a positive control. This reagent is a rabbit anti-human serum specific for class II antigens (24). Mini-pigs have a higher proportion of circulating B lymphocytes (which have class II antigens on their cell surface) than humans as well as having class II antigens present on their T cells (25,26). Thus, a reagent of this specificity could potentially give a positive reaction with unseparated T and B lymphocytes in mini-pigs. This anti-human serum reacted strongly with the mini-pig lymphocytes, making it a good positive control (26).

The SLA typing trays were set in the following format: well 1A contained the α P23,30, 1B contained FCS, and the rest of rows one and two contained the antisera at various dilutions. These rows were set in duplicate for each cell tested.

Other histocompatibility tests included SLA typing of all of the offspring of the NIH-identified generations, and the second generation which resulted from the unplanned breedings during the spring/summer of

1987. Table 1 shows the SLA types of the piglets from the planned matings in Boston. Table 2 gives an example of how these SLA types were assigned.

Lymphocytotoxicity Crossmatching. Crossmatching was done pre-transplant to verify an absence of anti-donor antibody in recipients prior to transplantation. This eliminated the problem of pre-formed antibodies which can lead to accelerated graft rejection. Only mini-pig pairs with negative crossmatches were used in this study. The crossmatches were performed in a similar manner to histocompatibility testing. The mini-pig serum being tested was heat-inactivated (56°C for 30 min) prior to use to destroy endogenous complement activity, and tested neat and at dilutions of 1:2, 1:4, 1:8, and 1:16 in RPMI with 10% FCS. Positive and negative controls were the same as described for histocompatibility testing. In addition to those, a row of RPMI-10% was added as a second negative control to assess background cytotoxicity.

The first column of the crossmatch tray containing neat test serum was left devoid of complement (5 μ l of RPMI-1650 were added to the column instead) as a control to determine whether cell death, when present, was due to complement-dependent antibody. The test sera and dilutions were run in duplicate. The results were scored according to the NIH-suggested technique, as described previously.

Autologous (auto) crossmatches were also performed in the same manner as the previously described crossmatch to assure the absence of detectable auto-antibody.

Mixed Lymphocyte Cultures (MLC). MLC were performed with pre- and post-transplant cells which had been cryopreserved, thawed, and washed prior to assay.

The cells were adjusted to 2.0×10^6 in RPMI-20% in duplicate sets of stimulators and responders. Mitomycin-C (25 $\mu\text{g/ml}$, final concentration) was added to each stimulator tube and the cells incubated at 37°C for 1/2 hr. Mitomycin C is added to inactivate cells by preventing DNA synthesis. These cells can serve as stimulators but cannot undergo cell division (27). The stimulator cells were then washed four times with RPMI-1640, resuspended in RPMI-20%, and adjusted to $0.5 \times 10^6/\text{ml}$. The responder cells were also adjusted to $0.5 \times 10^6/\text{ml}$ in RPMI-20%.

The MLC were performed in sterile 96-well microtiter trays (12 columns, 8 rows). Each stimulator was plated in triplicate columns, 100 μl of cell suspension/well. A control column of RPMI-20% was plated in triplicate as a negative control. Three columns were left for the addition of the T-lymphocyte mitogen phytohemagglutinin-A (PHA) as a positive control of the responder cells ability to proliferate.

The responder cells were added across the rows at 100 μl of cell suspension/well and one responder/row. A single row was left of stimulators alone for the addition of PHA on day three as a control to confirm the inhibitory effect of Mitomycin-C on the stimulator cells; they should not be able to proliferate in response to PHA. The MLC trays were then placed in humidity chambers in a 37°C incubator with 95% humidity and 5% CO_2 . PHA was added to appropriate wells at a concentration of 10 $\mu\text{g/ml}$ on day 3 of culture.

On day 6, the microtiter trays were pulsed with 50 μ l of 20 μ Ci/ml of tritiated thymidine (^{3} H TDR) and returned to the 37°C incubator for 6 hr. At the end of this period, the cells were harvested onto glass filter mats using a semi-automated cell harvester (Skatron). The mats were allowed to dry, the filter paper circles containing the cells punched out, and placed in 7 ml glass scintillation vials. Three ml of scintillation fluid were added to each vial and ^{3} H-TdR incorporation determined in counts per minute with a Beckman LS-330 scintillation counter.

The results of the MLC were analyzed by calculating the relative response of the responder cells; using the highest level of ^{3} H-TdR incorporated in one of three "unrelated" stimulators, which was assigned a 100% reactivity level (Figure 2). This procedure is recommended by the American Society for Histocompatibility and Immunogenetics (formerly the American Association of Clinical Histocompatibility Testing) as more accurate than using a pooled source of unrelated stimulators (14).

A-0 Typing. A-0 typing using anti-pig reagents was not performed at UT due to the unavailability of these reagents. As previously mentioned, the herd was reported to be all of type 0. Typing of pigs/mini-pigs with anti-human lectins had not been previously tried (personal communication, Dr. Joan Lunney, Beltsville, MD); however, several samples were randomly chosen and sent to the Blood Bank at UTMCK in a blind study for "ABO" testing. All samples were reported to be "0" (data not shown) on both forward and reverse typing.

Surgical Procedure. Mini-pigs from the herd were selected for transplantation according to their size, age, and SLA haplotypes. The

vascular transplants were performed simultaneously by two teams of surgeons from the University of Tennessee Medical Center at Knoxville and the University of Tennessee College of Veterinary Medicine. Sterile procedures were followed and the operations took place in the operating room at UTCVM Research Farm. The mini-pigs were sedated with ketamine and acepromazine, and anesthesia was induced using halothane in oxygen by mask. An endotracheal tube was placed and anesthesia maintained by halothane. Electrolyte fluids were administered intravenously during surgery. Transplants consisted of reciprocal exchange of the internal jugular veins as interposition grafts into the common carotid arteries of the recipients. Hence, each animal served as both donor and recipient. Transplants were performed in reciprocal pairs that were complete haplotype matches, one-haplotype matches, or complete mismatches. Each type of match was reciprocally transplanted on at least two occasions, yielding at least four pigs of each haplotype combination being transplanted. Post-surgical recovery took place in an isolation facility before the mini-pigs were returned to the colony.

Vascular Patency. Patency of the allografts was determined using a hand-held Medasonic Model BF4A Doppler flow detector. These procedures were performed by the staff of the Non-Invasive Vascular Laboratory at UTMCK. Blood flow in the transplanted veins was checked immediately post-surgery, then weekly until the veins were determined to be closed for two consecutive weeks. Graft closure was deemed indicative of graft rejection.

Statistics. Significance levels (p values) were determined by using either the Fisher's Exact Test or the student's T-test. The

T-test is used when comparing means from two populations to determine if they are different. The Fisher's Exact Test gives the exact probability that something is or is not significant, similar to a Chi Square Test. These tests measure the association between two factors or sets of data by determining the observed frequency of the distribution of the actual data versus the expected distribution assuming there is no association.

CHAPTER 3

RESULTS

A. SEROLOGIC ASSAYS

SLA Typing. The SLA tissue typing results of the mini-pigs used in these studies and donor/recipient pairs are shown in Table 3. There was complete agreement between the SLA haplotype assignments, as designated by the NIH, and those retyped at UT.

Crossmatching. The selection of mini-pig pairs for the transplants was done by SLA type, size, age, and absence of SLA or autoantibody, as determined by lymphocytotoxic crossmatching, as discussed in the Methods section. Not only were the appropriate combinations of SLA haplotypes transplanted, but the physical aspects of the surgery were less complicated when dealing with animals of similar size.

Two recipients were lost due to post-surgical complications. The first recipient died post-transplant due to undetermined causes. The second never recovered from anesthesia. Antibody development after transplantation in the remaining recipients is shown in Figure 3. In the SLA completely matched group, one out of five recipients developed lymphocytotoxic antibody to its donor, as detected by crossmatch analysis at week three. All other recipients in this group remained crossmatch negative to their donor through week 14. All four recipients in the 1-haplotype matched group developed anti-donor antibody, occurring from one to six weeks post-transplant (group mean = 3.25 wks). In the completely mismatched group, three out of four recipients developed

anti-donor antibody, two at week two and one at week three. The fourth still had not developed antibody at week nine (group mean = 2.3 wks).

In order to test the specificity of the antibodies that developed post-transplantation, all positive post-transplant sera were tested for cytotoxicity against a panel of SLA-typed cells. The cells selected for the test were homozygous at their SLA loci: AA, CC, or DD. The sera were those in which antibody was detected by the crossmatch test. All sera were tested neat through to 1:16 in the standard histocompatibility testing assay described in the Methods section of this thesis. The results are shown in Table 2. Sera 1677 and 1635 showed reactivity against the appropriate cells in the panel of SLA-defined lymphocytes. Recipient 1677 (an AC) was transplanted with an AD; therefore, one would expect to see anti-D antibody exclusively, which is exactly what occurred. Recipient 1635 (an AD) was transplanted with an AC, and developed the appropriate anti-C antibody. Sera 140 and 1535 showed cytolytic activity against all AA and CC cells. Recipient #140's SLA type was AD, as was its donor; thus, these antibodies are probably non-SLA. Recipient #1535's SLA type was DD, its donor's type was CC. Recipient #1535 developed the expected anti-C antibody, but also demonstrated reactivity against all of the AA cells on the panel. Mini-pig #1686 developed only anti-D, as expected; the recipient being an AA and the donor a DD. Recipient #1709, a DD, developed anti-A; the donor having been an AA. Recipient #1709 also reacted against the CCs on the panel and one of three DDs, an unexpected finding. Mini-pig #148, an AC, developed anti-D, the donor having been a CD. Recipient #1646, a CD, developed the expected anti-A; its donor having been an AC.

B. GRAFT PATENCY

Graft survival varied among the three types of SLA-matched groups, as determined by following graft patency by Doppler analysis. If there was no blood flow detectable for two consecutive weeks, the veins were declared closed and deemed rejected. As shown in Figure 4, in the SLA-completely matched group, grafts remained open for four, seven, nine, and greater than 16 weeks (group mean = 9.0 wks). The 1-haplotype matched group had shorter graft survivals, with grafts closing on weeks two, six, and seven (group mean = 5.0 weeks). Completely mismatched recipients had grafts which closed on weeks three or four and a mean graft survival of 3.5 weeks.

In comparing the completely matched group to the mismatched groups, graft closure occurred within two weeks of antibody development in the mismatched groups, regardless of whether they were a 1-haplotype match or a complete mismatch. Seven out of eight of the mismatched recipients developed antibody and all lost their grafts, whereas only one out of five of the completely matched grafts developed antibody but it retained its graft for >16 weeks. The completely matched grafts also lasted an average of 4.75 weeks longer than the mismatched grafts. The significance of these findings appears to lie in the fact that in completely matched recipients, antibody development and graft rejection do not correlate. On the other hand, SLA mismatching had a direct impact on antibody production and graft survival.

C. CELLULAR ASSAYS

The serologic results described directly measured B-lymphocyte responses of the recipient in the form of anti-donor antibody production. However, T lymphocytes are also frequently activated by histocompatibility differences. Hence, it was also important to assess T-lymphocyte activation in transplant recipients.

Accordingly, the MLC was performed on the mini-pigs pre- and post-transplant. This assay primarily measures incompatibility at the class II region by T-lymphocyte proliferation. An example of an MLC is given in Table 5 and complete results for this project are shown in Figure 5.

There were six mini-pigs in the SLA-completely matched transplant group. Of these six, three had a relative response of 0% pre-transplant, and the remaining three had relative responses of 4, 8, and 9% (mean = 3.5%). The 1-haplotype matched group had relative responses of 0, 5, 25, and 100% pre-transplant (mean = 32.5%). The completely mismatched group had relative responses of 15, 36, 61, and 63% (mean = 41.25%).

Post-transplant MLC were performed using lymphocytes collected at the time when anti-donor antibody production was first detected. In those recipients failing to develop antibodies, two arbitrary dates comparing the median and end point of the study were chosen.

The highest relative response obtained from the completely matched group was 71% at week three post-transplant for mini-pig #179, who failed to develop anti-donor antibodies. The next highest response in this group was 23% at week four for #1587, who also failed to develop anti-donor antibody. Recipient #140, who developed a positive

crossmatch, showed no change in MLR post-transplant. The mean of the group was 12.2%, the range being from 0 to 71%; peak responses being reached by week nine. #1707 died post-transplant before any further MLCs could be performed.

One hundred percent relative response was reached by one-half of the recipients in the 1-haplotype matched group, the range being 58-100% (mean 79.5%). The peak responses were reached by week three in all four recipients in this group.

In the completely mismatched group, three of four recipients achieved a 100% relative response against their donors. These peaks were established by week four, the mean relative response being 79.0%. One recipient (mini-pig #1686) in this group was a poor responder, having on three occasions given relative responses of 15% (pre-transplant), 16%, and 0%, respectively.

CHAPTER 4

DISCUSSION

Vascular transplants were performed in miniature swine to assess the effects of the MHC on antibody induction, graft survival, and T-cell mediated immunity. An overall summary of the results obtained is depicted in Table 6. Antibody development was directly influenced by the degree of SLA match. In the mini-pigs transplanted with partial or no match, six of seven recipients developed antibody, as opposed to only one out of five of the completely matched donor-recipient pairs ($p = 0.44$ by Fisher's exact test). Notably, antibody developed preceded or coincided with graft closure in mismatched recipients.

Antibody development in the recipients was shown to be not only anti-donor antibody but, in mismatched recipients, was frequently SLA specific (Table 4). The cells used in the serological assays were not enriched for either T or B lymphocytes. Since both populations carry class I antigens on their cell surfaces (28), the antibodies are presumed to be anti-class I. Activated T lymphocytes in the human (29) and certain subpopulations of T lymphocytes in mini-pigs are known to carry class II antigens, as well as B lymphocytes and macrophages (10). Some of the antibody in the post-transplant sera could be class II, but this was not determined during this study. The strength of reactions in the microlymphocytotoxicity assays indicated the presence of class I antibody, since 100% cell lysis was achieved in the post-transplant cross-matches and in the majority of the assays testing these sera for SLA specificity.

Five recipients (#1677, 1635, 1686, 148 and 1646) developed the expected SLA specificity exclusively. More complex were the sera from mini-pigs #140, #1535 and #1709, which showed extra reactivity against the homozygous cell panel. Recipient #140 was an AD genotype and was transplanted with an AD. This mini-pig should not have developed anti-SLA antibodies; thus, they were possibly non-SLA directed. Another possibility for the antibody development in recipient #140 is that the donor (#144) could be a recombinant for SLA loci. The typing sera used recognized haplotypes, not individual SLA products. Therefore, #144 could still type in a serological assay as an AD due to appropriate AD class I antigens, when in fact the class II antigens may be inappropriate for the AD haplotype. If so, the antibody in #140 following transplantation with #144's graft may be class II directed, which could give a strong reaction in a lymphocytotoxicity assay due to the fact that class II is found on T lymphocytes in swine, as well as on B lymphocytes (9,25,26). These possibilities could not be distinguished using the techniques employed in this project. One way to examine the possibility of the serum having anti-class II would be to absorb it with cells lacking class II antigens, such as platelets. The serum could then be retested in the lymphocytotoxicity assay and analyzed for remaining reactivity, which would be class I specific. Recipient #1535 was a DD genotype transplanted with a CC graft. Anti-C was present as expected, but anti-A was also detected. This extra reaction could be due to the fact that there is crossreactivity in the SLA system, due to "public" specificities shared by distinct haplotypes in addition to "private" specificities (30). A public MHC antibody is one that

recognizes a portion (epitope) of the antigen shared by more than a single haplotype, not the epitope(s) defining the private specificity (37). A means to test for this in recipient #1535's serum would be to perform absorptions with AA cells and determine what reactivity is removed from the serum. If all reactivity is removed, then the serum probably contained only a public SLA antibody; if only AA reactivity is absorbed (i.e., C reactivity persists), then the serum is probably a mixture of public and private specificities.

Haplotypes C and A have been reported to share histocompatibility specificities, and haplotypes D and A seem to share public Ia specificities (31). The C-A crossreactivity may explain the anti-A developed by #1535. Recipient #1709 (DD) was transplanted with an AA and showed anti-A and anti-C antibody development, again possibly due to public crossreactivity. This recipient also reacted to one of three DD cells, possibly indicating a non-SLA antibody as well. Absorption assays might be used to distinguish these possibilities. For example, if serum from recipient #1709 was absorbed with cells homozygous for the "A" haplotype and all reactivity was removed from the serum, the specificity would be against a public determinant rather than just the private determinant of "A." If "C" cells were used for absorption, the "A" specificity would remain in the serum if only a private "A" antibody existed. This would also happen when using "D" cells. If all reactivity was removed by any of these cells, one would have to conclude that a public specificity was indeed present that is shared by each of these antigens.

In summary, from the serologic analyses, it appears that antibody development is dependent on the degree of SLA matching. If an SLA

mismatch is present, the humoral immune system is usually activated, producing anti-donor antibodies to the mismatched antigens.

The influence of the MHC on T-cell responses was evident among the three groups of mini-pigs receiving transplants with different degrees of SLA matching. In the completely-matched group, one would not expect to see any significant stimulation since the donors and recipients were identical for class II antigens. Fifteen percent was the minimum relative response used as the cutoff for MLC being considered positive. All completely matched pairs fell below this range before transplantation. The stimulation present in this group could possibly be attributed to non-SLA antigens, which can lead to low stimulation rather than the higher levels caused by SLA class II differences (32,33). There was no significant difference in these recipients between pre- and post-transplant MLC ($p > 0.05$; one-tailed paired t-test). The 1-haplotype matched group showed a significant difference between their pre- and post- MLC ($p < 0.05$, one-tailed paired t-test). The mean relative responses went from a pre-transplant value of 32.5% to a post-transplant value of 79.5%. Completely mismatched transplant pairs also showed a significant increase in relative response post-transplant. The pre-transplant MLC gave a mean relative response of 41.25%; whereas the post-transplant MLC gave a relative response of 79% ($p = < 0.02$, paired t-test). Thus, a strong correlation is observed between the degree of SLA matching and the strength of T-cell responses in the MLC, consistent with the immune response to non-identity at the class II region of the MHC.

Graft patency was also directly affected by the degree of SLA match. Even though there was a difference between complete, 1-haplotype, and complete mismatch groups for the length of time their grafts remained open, the difference between 1-haplotype and complete mismatches was not considered significant ($p > 0.05$). The biggest difference was between completely matched grafts and all the others combined: mean = 9.0 weeks versus 4.2 weeks, respectively ($p < 0.02$; t-test). These results agree with other studies in that a complete match gives the best results in terms of delaying graft rejection (7,10,11,16).

Antibody development and graft rejection were not clearly linked to each other. In the completely matched group, one of the five recipients developed antibody; however, its graft was still open at week 16 post-transplant. Conversely, of the three recipients in this group whose grafts did close, none developed lymphocytotoxic antibody. This seems to indicate that, in the completely matched group, antibody development and graft rejection did not coincide or that undetected antibody responses were directed against allograft antigens which are not expressed by lymphocytes, e.g., endothelial-specific antigens (34). This was not the case in the 1-haplotype and completely mismatched recipients who usually developed anti-donor SLA antibody. In the 1-haplotype group, all developed antibody and all rejected their grafts. In the completely mismatched group, three out of four developed SLA antibody and all rejected their grafts. In this group, mini-pig #1698 failed to show antibody development when crossmatched against its donor even though its graft rejected. It is well known that high titered antisera may fail to

"react" at low dilutions due to the prozone effect (35,36). In the literature, SLA sera were reported with antibody titers of 1:512 after skin grafting (6), and 1:1024 when tested in our laboratory as SLA-specific antisera. With this knowledge, serum from #1698 was retested in two-fold dilutions to 1:1024 to test for possible prozone effects. The serum remained inactive, verifying a lack of detectable cytotoxic antibody. Possible reasons for the apparent lack of antibody in #1698 could be due to any of several reasons. The humoral system may not be functioning correctly, due to a problem with antigen-presenting cells, or failure of B lymphocytes to differentiate properly (38). Other possibilities for a lack of detectable antibody could be that antibody is being produced but either in insufficient amounts to bind complement or that it is a non-complement fixing antibody. A more sensitive technique could be employed, such as fluorescent staining or an indirect antiglobulin technique which facilitates complement fixation (39).

Another possibility is that a blocking antibody may have been formed which is keeping the anti-donor antibody from attaching to the antigens on the cell surface, thereby prohibiting complement fixation and cell lysis. Anti-idiotypic antibodies can block antigen-antibody reactions by attaching to the anti-donor antibody, thereby preventing the latter from attaching to the donor antigens on the cell surfaces (40). This would give a negative result in a microlymphocytotoxicity assay.

This result, coupled with graft rejections observed in completely matched recipients without detectable antibody, indicated that antibody may not be necessary for graft rejection. These results agree with

those of Kirkman, et al., who described this phenomenon when examining mechanisms of hyperacute or accelerated graft rejection of renal transplants in "pre-sensitized" mini-pigs.

As seen with antibody formation and graft survival, there was no apparent correlation in the completely matched group between antibody formation and cellular response. Two of the five mini-pigs developed a positive MLC (>15% cutoff) post-transplant; yet, neither of these developed detectable antibody and only one rejected its graft. Conversely, mini-pigs in the mismatched groups all developed a positive MLC, and seven out of eight developed antibodies. One of this group reached 100% relative response yet failed to produce antibody. All the mini-pigs in the mismatched group which developed antibody did so within two weeks preceding graft rejection. These results suggest that antibody development is an indicator of graft rejection in the SLA mismatched groups, but not in the matched groups. This could be due to sample size or to the criteria used to establish graft rejection. Serial studies of antibody detection combined with cellular assays may be better indicators of graft rejection and give a better correlation between these assays and graft rejection.

When comparing graft patency and cellular response, the following observations were made. In the completely matched group, of the two mini-pigs whose grafts remained open, one had a negative MLC both pre- and post-transplant, and the other had a pre-negative and post-positive MLC. Of the three remaining recipients in this group, all had closed grafts, yet only one had a positive MLC and it was weak (RR = 23%). All of the recipients in the 1-haplotype group had closed grafts; one of

three had a strongly positive pre-transplant MLC and all had positive post-transplant MLCs. In the completely mismatched group, all had closed grafts and three out of four developed increasingly reactive MLR. As described previously, #1686 was consistently a low-responder in MLC. Extraneous reactivity or non-reactivity in the MLC could be due to the reproducibility of this test. Since the relative responses are based on the highest non-related stimulator, this cell should always be included in an assay for that particular pig, as well as the other controls being consistent. This was not always possible due to a lack of cells from certain animals. The MLC was repeated in several pairs of donor/recipients when aberrant results were obtained to control for these possibilities. The length of time the cells were frozen could also have an impact on their responsiveness or ability to simulate in an MLC. This has not been explored.

A possibility for recipient #1686's low levels of response in the MLC could be due to an immune response defect. The T lymphocytes may not be able to recognize foreignness in a mismatched class II antigen; therefore, an immune response would not be mounted and the cells would fail to respond and go into blastogenesis. The T lymphocytes may recognize the foreignness, but a defect may exist in cell differentiation which would also prohibit an immune response from taking place.

The post-transplant MLC was performed at the time of antibody development, at a midpoint, and at the time of graft closure, to determine if an increase in reactivity could be detected. The level of relative response increased in the majority of the mismatch recipients (Figure 5). These results suggest that the strength of the MLR does

predict with grafts will be rejected most rapidly in parallel with the degree of SLA mismatch at the class II region.

In summary, the serological and cellular assays performed agree with results obtained in other transplant models in the mini-pig, as well as those of the HLA and DLA systems. According to Calhoun et al., the degree of histocompatibility matching affected the rate of allograft failure but not necessarily the occurrence in dogs. He found that matched animals can still reject their grafts but at a much slower rate than the mismatched animals. These findings agree with our graft failures for zero, 1-haplotype and completely mismatched groups. Most of these recipients lost their grafts but more rapidly with greater mismatching. The serological aspects of this project agreed with those of Leight et al., along with others, who tested post-transplant sera from skin allograft recipients against a panel of SLA-typed lymphocytes and found post-transplant antibody to be donor-specific, plus the additional correlations of anti-SLA and public or possibly crossreactive antibodies (Table 4). In agreement with Kirkman et al., the MLC could be used to predict the most rapid rejectors as demonstrated by our mismatched recipients, but could not be used to predict rejection in the completely-matched group.

The interrelationships of the various aspects of transplant immunology obtained in these experiments are shown in Figure 6. The bold arrows indicate a strong correlation, the lighter arrows indicate a weaker correlation. Mismatching at the MHC does have a direct effect on antibody production, rate of graft rejection and strength of the T-cell

response; whereas a much weaker correlation was observed between antibody production, cellular responses and graft rejection.

The mismatched grafts were rejected more rapidly than the matched grafts; in fact, all were rejected by week seven post-transplant. The cellular response was detected earlier and was stronger in mismatched grafts than matched grafts. The humoral response was stimulated earlier in mismatched grafts than in matched grafts. All of these areas of transplant immunology are directly affected by the MHC. The correlations between each of the above mentioned areas to one another are not as strong, but do exist, especially among mismatched recipients. The order in which the experiments were performed in this project could not detect which may have come first: cellular or humoral immune responses, or if they occurred simultaneously. It has been shown that the immune responses precede rejection in other transplantation models; therefore, it appears that maximum matching for the MHC is desirable for vascular transplantation.

In future studies in this model, additional donor/recipient cells should be banked so sequentially obtained recipient samples could be repeatedly tested simultaneously using the same set of controls. Auto-grafts need to be performed, as do attempts to perform the surgeries using the same personnel and, whenever possible, as many pairs of donor/recipients at the same time to control for the variations in surgical technique among various surgeons, and to avoid the possibility of the technique improving with time rather than the SLA matching having a major effect on graft survival.

Since it has been shown that the MHC (in this case, SLA) is a very important factor for graft survival, the next step should be to look at manipulation of the transplant recipients with immunosuppression and of the vessels through cryopreservation and endothelial stripping. Cryopreservation has been shown to make vessels less antigenic when tested in small animal models, as has removing the endothelial lining of the vessels by collagenase digestion (1,4).

Another factor to consider when performing vascular allograft transplantation is the vascular endothelial cell (VEC) antigen system. It has been shown in the human that non-HLA antibodies have been identified which react specifically with non-HLA antigens on the vascular endothelium (34). The possibility of this type of antigen system existing in the mini-pig and being able to induce an immune response leading to graft rejection needs to be further explored. The presence of non-HLA/SLA antigens present on the vascular endothelium may have as much influence on vascular allograft survival as the HLA/SLA antigens, as has been shown in this study.

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APPENDICES

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APPENDIX A

TABLE 1

ASSIGNMENT OF SLA HAPLOTYPES OF OFFSPRING FROM PLANNED MATINGS

PARENTAL		
HAPLOTYPES	OFFSPRING #	OFFSPRING HAPLOTYPES
AA X AC	148	AC
	149	AC
	150	AC
AC X AC	182	AC
	183	AA
	185	CC
AC X AC	177	AC
DD X AD	140	AD
	143	AD
	144	AD
	146	AD

The SLA types detected on "adolescent" mini-pigs which had not been previously typed. Column one shows the SLA types of the boars and sows. Column two is the identifying number given to each mini-pig. Column three is the assigned SLA haplotypes.

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TABLE 2

SELECTION OF SLA HAPLOTYPE ACCORDING TO REACTIVITY TO KNOWN SLA ANTIGENS

PARENTS	OFFSPRING #	CYTOLYTIC SCORE			ASSIGNED	
		ANTISERA #2331	#1921	#1919	SLA	TYPE
		SPECIFICITY α A	α C	α D		
1674 (AC boar), 1672 (AC sow)	182	8	8	1	AC	
	183	8	1	1	AA	
	185	1	8	1	CC	
1536 (DD boar), 1633 (AD sow)	140	8	1	8	AD	
	143	8	1	8	AD	
	144	8	1	8	AD	
	146	8	1	8	AD	

The reaction patterns given by the various SLA-typing sera and the previously untyped mini-pigs. Column six gives the assigned SLA types, based upon their lymphocytotoxicity reaction patterns. Strength of cytotoxicity is indicated by the NIH-suggested technique: 8 = 90-100% cell lysis; 1 = 0-10% cell lysis.

TABLE 3

SLA HAPLOTYPE ASSIGNMENTS OF THE MINIATURE SWINE

CHOSEN FOR TRANSPLANT

MINI-PIG #	NIH ASSIGNMENT	UT ASSIGNMENT	DONOR
1535	DD	DD	1698
1587	DD	DD	1707
1615	DD	DD	
183	AA	AA	
1686	AA	AA	1709
149		AC	
1672	AC	AC	
177		AC	
182		AC	
1677	AC	AC	1635
148		AC	1646
150		AD	
1635	AD	AD	1677
140		AD	144
143		AD	
144		AD	140
1698	CC	CC	1535
179		CC	185
1646	CD	CD	148
1707	DD	DD	1587
1709	DD	DD	1686

The SLA haplotype assignments as designated by the NIH and, in adults (4-digit numbers), verified at UT. The 3-digit numbers are offspring which had not been previously typed.



— QUALITY —

	SLA	CELL										OBSERVED
		DONOR	RECIP.	2-1	183	1686	1698	179	3-3	1709	1587	
		AA	AA	AA	AA	CC	CC	CC	CC	DD	DD	SPECIFICITY
1677	AD	AC	-	-	-	-	-	-	-	+	+	D
1635	AC	AD	-	-	-	NT	+	+	+	-	-	C
140	AD	AD	+	+	+	+	+	+	+	-	-	non-SLA
1535	CC	DD	+	+	+	+	+	+	+	-	-	CC + public
1686	DD	AA	-	-	-	-	-	-	-	+	+	D
1709	AA	DD	+	+	+	+	+	+	+	-	-	A+
148	CD	AC	-	-	-	-	-	-	-	+	+	D
1646	AC	CD	+	+	+	-	-	-	-	-	-	A

The reaction patterns of the post-transplant sera of those recipients that developed antibody. The samples are from crossmatches which detected anti-donor antibody. The known SLA-typed cells are in the columns; the sera are in the rows.

TABLE 5

MLC RESULTS: MEAN CPMs AND RELATIVE RESPONSE

STIMULATOR	1535x (DD)	1587x (DD)	1709x (DD)	1672x (AC)	ADKINS	MEDIA	PHA
1535 (DDR)	407	831	7639	68309	12832	262	46102
	0	0	11	100	18	0	67
1587 (DDR)	352	558	6253	37529	11642	698	94204
	0	0	15	100	30	0	253
1709 (DD)	1290	1612	5140	63590	10603	3972	68627
	0	0	0	100	9	0	108
1672 (AC)	6870	12736	19115	1425	37975	530	51726
	30	64	100	0	207	0	284
Adkins	60357	43379	42118	68943	6180	2267	57408
	86	59	57	100	0	0	82
PHA	108	132	652	183	326		

The results of an MLC. The columns represent stimulator cells and their SLA haplotypes. The last two columns are negative and positive controls. The rows represent the responder cells. The last row contains PHA as a control of inhibition of the stimulators. Two values are given for each set of responder/stimulators: The top numbers are the mean cpm and the bottom numbers are the relative response.

TABLE 6

TRANSPLANT RESULTS: CROSSMATCH, GRAFT SURVIVAL AND MLR

PIG #	SLA MATCH	CROSSMATCH	GRAFT CLOSURE	MLR	
				PRE	POST
140] 144]	Complete Complete	3 -	- (>16) 9	8 9	2 9
1587] 179]	Complete Complete	- -	4 - (> 9)	4 0	23 71
185] 1707]	Complete Complete	- (Died post-tx)	7 NT	0 0	3 NT
1677] 1635]	One-Half One-Half	1 1	2 NT (Surgical Mishap)	5 100	100 100
148] 1646]	One-Half One-Half	5 6	7 6	0 25	60 58
1535] 1698]	0 0	3 -	3 4	63 51	100 100
1686] 1709]	0 0	2 2	3 4	15 36	16 100

Column one gives the recipient's number; column two the type of SLA match; column three the crossmatch results in weeks; column four is graft closure in weeks, and columns five and six give percent RR of the MLR. Brackets designate transplant pairs.

APPENDIX B

SWINE: SLA COMPLEX

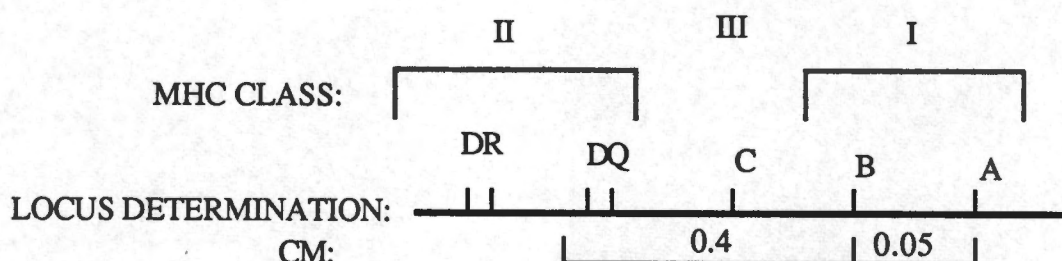


Figure 1. Structure of the SLA complex. Letters above the line designate individual loci. Roman numerals above letters indicate which class of MHC antigen is coded by the locus. The lowest line designates apparent recombination distance, in centiMorgans (cM), between loci for which data is available (35,39). The relative position of different loci within each class is unknown for SLA; positions shown are based on those reported for the HLA complex. (Figure reproduced with permission of the author, [6].)

$$\frac{\text{EXPERIMENTAL MLC} - \text{AUTOLOGOUS MLC}}{\text{REFERENCE VALUE} - \text{AUTOLOGOUS MLC}} \times 100$$

Figure 2. Relative Response. The formula is to determine the relative response in an MLC. The reference value is equal to the highest unrelated responder minus the autologous control.

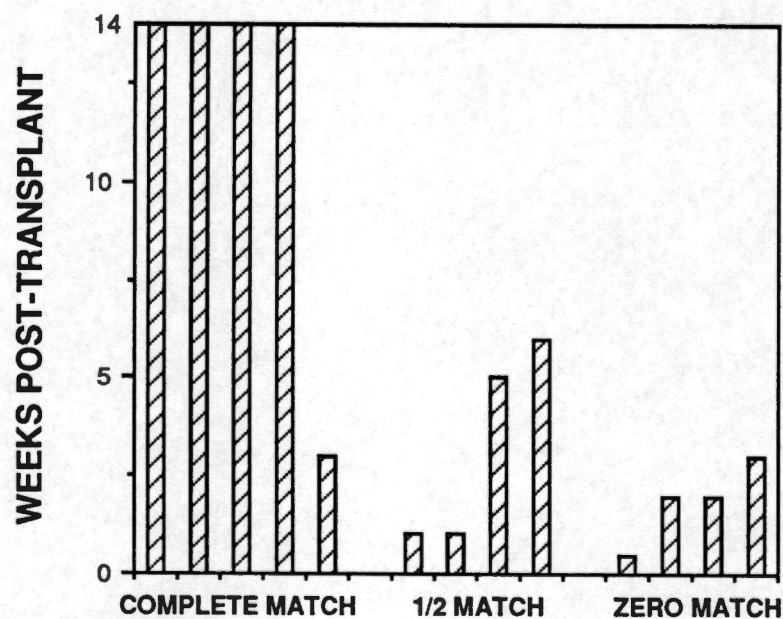


Figure 3: Development of Antibody in Each of the Mini-Pigs Following Transplantation. The recipients are grouped according to SLA match and each bar represents a single recipient. The bars end at the week antibody was first detected or at termination of the study, whichever came first.

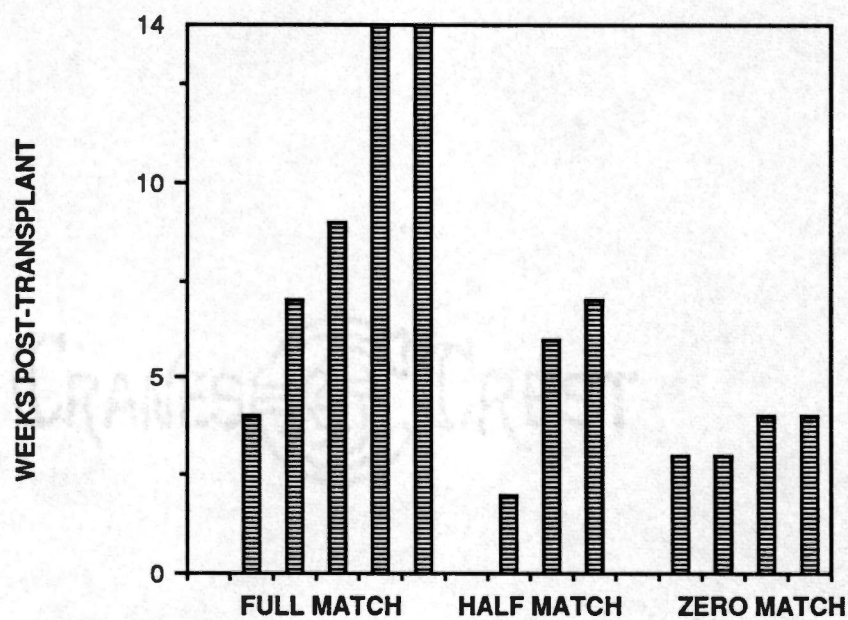


Figure 4. The Length of Graft Survival in Mini-Pigs Versus Their SLA Match. Two of the recipient grafts in the completely matched group were still open at week 16 of the study.

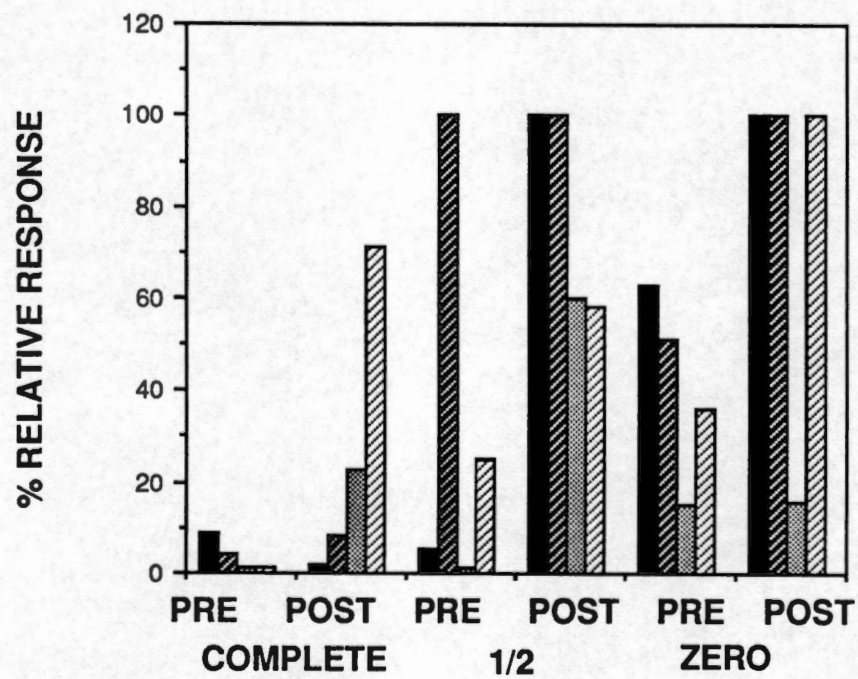


Figure 5: The Percent Relative Response in Each of the Transplant Groups, Pre- and Post-Transplant. Individual recipients' pre- and post-transplant responses are indicated by bars with like shading.

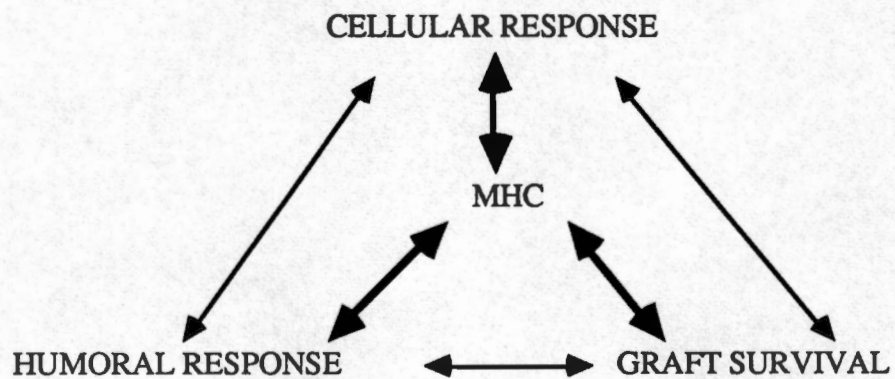


Figure 6:.. The MHC and Its Effect on the Various Aspects of Transplantation.

VITA

Joan E. Bray was born in Norman, Oklahoma. She was raised in Pine Beach, NJ, and graduated from Toms River High School South in 1971. Joan received a Bachelor of Science degree from Trenton State College in 1975, with a major in health sciences and minors in biology and psychology.

From 1975 until 1984, Joan worked at the Tissue Bank of the Naval Medical Research Institute, Bethesda, MD. During this time, she took graduate classes in immunology at the Foundation for Advanced Education in the Sciences, the National Institutes of Health, Bethesda, MD.

In 1984, Joan moved to Knoxville, TN, to help establish and supervise a histocompatibility testing laboratory at the University of Tennessee Medical Center at Knoxville. At this time, she also started graduate studies in the Comparative and Experimental Medicine program, pursuing a Master's of Science degree.