Large Animal Models

for assessing immune response and immune tolerance

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Large Animal Models

Large size/long life span may be important for certain applications

- Cellular constructs with scaffolds (too big for rodents)
- Mimic clinical conditions of tissue damage as well as tissue repair/regeneration (Surgical model)
  - Cardiac infarct model
  - Subretinal injections
- Long term follow-up of individual animals
  - Multiple biopsies possible
    - Not necessary to sacrifice animals at each time-point for proper assessment
  - Long-term effects of treatment can be determined
    - Toxicity studies
    - Repeat injections of cellular therapy

Large animal models for biomedical research

Non-Human Primate, Sheep, Dog, Pig

- NOT useful for testing immunogenicity of human cells/cellular constructs
  - Xenogeneic barriers – NK cells, Mϕ
    - CD47: CD172 (SIRPα) incompatibility
      - Yang YG. CD47 in xenograft rejection and tolerance induction 2010 *Xenotransplantation* 17(4):267-73
- Need to develop an analog of the human cells/constructs for each species and test in autologous setting or across MHC barriers depending on the clinical application

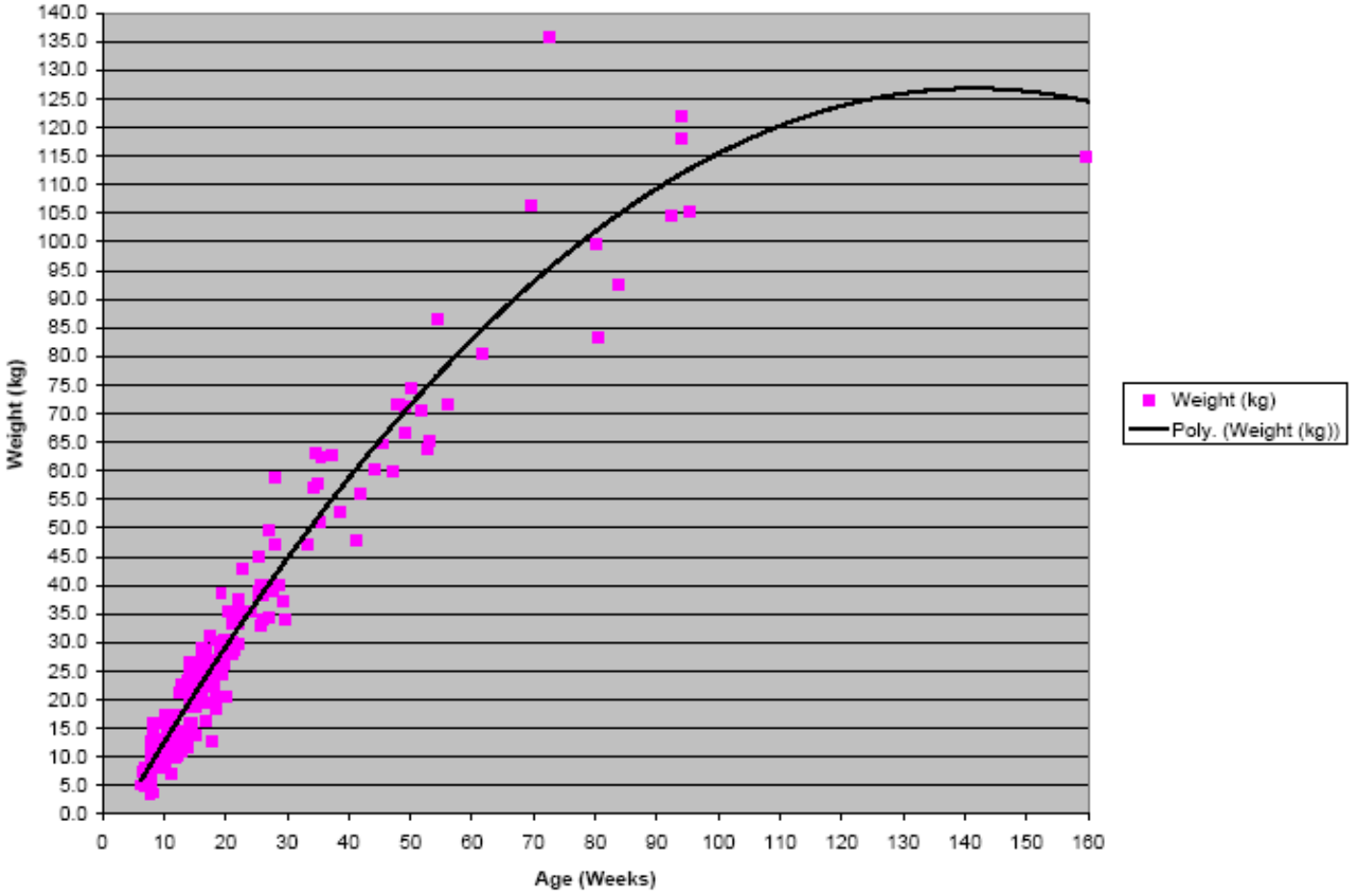
Miniature Swine

- Size range similar to humans (10-125kg)
  - Omnivore physiology similar to humans
- Extensively used in biomedical research
  - Porcine specific reagents available
    - Numerous phenotypic markers to assess immune response
      - Haverson et al. Overview of the Third International Workshop on Swine Leukocyte Differentiation Antigens. Vet Immunol Immunopathol. 2001 Jul 20;80(1-2):5-23.
    - Markers to distinguish donor from host cells available for certain pig strains
      - Pig Allelic Antigen (PAA)
        - Fuchimoto et al. Tissue Antigens. 1999; 54: 43-52.
      - CD4 allele specific monoclonal antibody
      - SLA specific monoclonal antibodies

Miniature Swine

- Pig skin similar to human skin
  - Low hairiness, thick stratum corneum, similar lipid composition, dermis structure
    - Useful for trans-dermal vaccine development
    - Useful for assessing skin substitute biologics
- Responses to BMT similar to patients
  - Manifestations of Graft-versus-Host Disease (GvHD) – similar grading
  - Complications of post transplantation lymphoproliferative disorder (PTLD)
    - Porcine γherpesvirus involvement (PLHV-1)

Miniature Swine
Average Weight versus Age



MGH MHC-Defined Miniature Swine

X

Haplotype (SLA)	Origin of Regions: Class I	Class II
A	a	a
C	c	c
D	d	d
G	c	d
H	a	d
J	a	c
K	d	c
L	d	a

Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. Sachs DH, Leight G, Cone J, Schwarz S, Stuart L, Rosenberg S. Transplantation. 1976 Dec;22(6):559-67.

MGH MHC-Defined Miniature Swine

Clinical Situation	***Miniature Swine Model***
HLA identical sibling transplants	SLA-matched haplotypes
Cadaveric or non-matched siblings	SLA-disparate haplotypes
One-haplotype mismatched sibling transplants or parent into offspring transplants	One-haplotype mismatched heterozygotes (haploidentical) (SLA^{ac}->SLA^{ad})

DERIVATION OF SLA^{dd} INBRED SUBLINE

HISTOCOMPATIBLE MINIATURE SWINE: AN INBRED LARGE-ANIMAL MODEL[1]

JOSHUA D. MEZRICH,[2] GARY W. HALLER,[2] J. SCOTT ARN,[2] STUART L. HOUSER,[2,3] JOREN C. MADSEN,[2,4] AND DAVID H. SACHS[2,5]

Three herds of miniature swine, each homozygous for a different set of alleles at the major histocompatibility complex (MHC), and five intra-MHC recombinant strains, have been reported by the authors' laboratory. One herd (SLA^{dd}) was selected for further inbreeding to achieve a histocompatible line. It has undergone seven additional generations of sequential brother-sister or father-daughter matings (termed *G7*). To determine the level of histocompatibility of these animals, the authors performed skin and heart transplantation without immunosuppression. In contrast to MHC-matched, minor antigen-mismatched animals that rejected skin in 11 days (median survival time [MST], n=6) and hearts in 35 days (MST, n=4), G7 animals accepted skin for greater than 340 days (>340, >448, and >677 days)

[1] This work was supported in part by grants from the National Heart, Lung, and Blood Institute of the National Institutes of Health (PO1 HL18646-24 and RO1 HL54211-05).

[2] Transplantation Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

[3] Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

[4] Division of Cardiac Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Transplantation 2003 vol. 75 no. 6 744-749.

Currently maintaining G11 animals with coefficient of inbreeding >95%
Recent data confirms skin and lung graft acceptance without immunosuppression

Adoptive transfer studies in large animals now possible

Immunogenicity Studies in MGH MHC-Defined Miniature Swine

BLOOD, 1 JANUARY 2008 • VOLUME 111, NUMBER 1

Immunogenicity of umbilical cord tissue–derived cells

Patricia S. Cho,[1] Darin J. Messina,[2] Erica L. Hirsh,[1] Nina Chi,[2] Stephanie N. Goldman,[2] Diana P. Lo,[1] Ian R. Harris,[2] Sicco H. Popma,[2] David H. Sachs,[1] and Christene A. Huang[1]

[1]Transplantation Biology Research Center, Massachusetts General Hospital, Boston; and [2]Centocor R&D, Stem Cell Internal Venture, Radnor, PA

(*Transplantation* 2010;90: 494–501)

Approaches to Avoid Immune Responses Induced by Repeated Subcutaneous Injections of Allogeneic Umbilical Cord Tissue-Derived Cells

Bram V. Lutton,[1] Patricia S. Cho,[1] Erica L. Hirsh,[1] Kelly K. Ferguson,[1] Alexander G. S. Teague,[1] John S. Hanekamp,[1] Nina Chi,[2] Stephanie N. Goldman,[2] Darin J. Messina,[2] Stuart Houser,[3] Beow Y. Yeap,[4] Sicco H. Popma,[2] David H. Sachs,[1] and Christene A. Huang[1,5]

Immune Response Testing in Miniature Swine

Naïve SLA-matched bleeder pigs are available from each haplotype for assay controls.

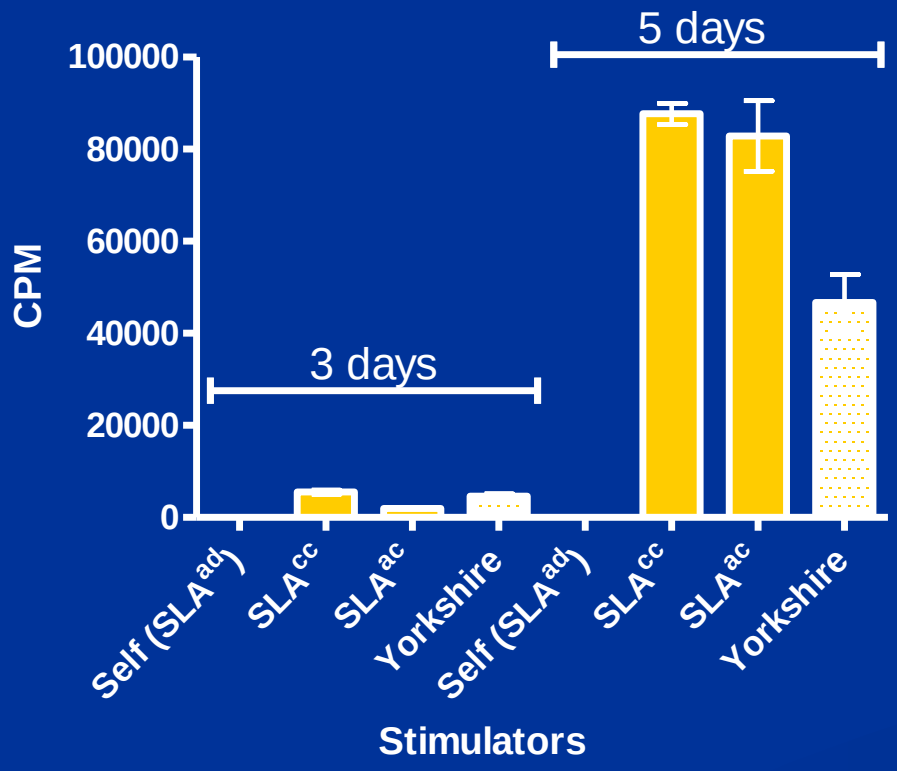
Recombinant haplotypes are available to distinguish responses to MHC class I vs class II.

- Mixed Lymphocyte Reactivity (MLR)
  - CFSE and H^3 Thymidine incorporation
- Cell Mediated Cytotoxicity (CML)
- Donor specific antibody responses
  - Serum antibody binding to PBMC of different haplotypes
  - Complement dependent antibody mediated cellular cytotoxicity assay

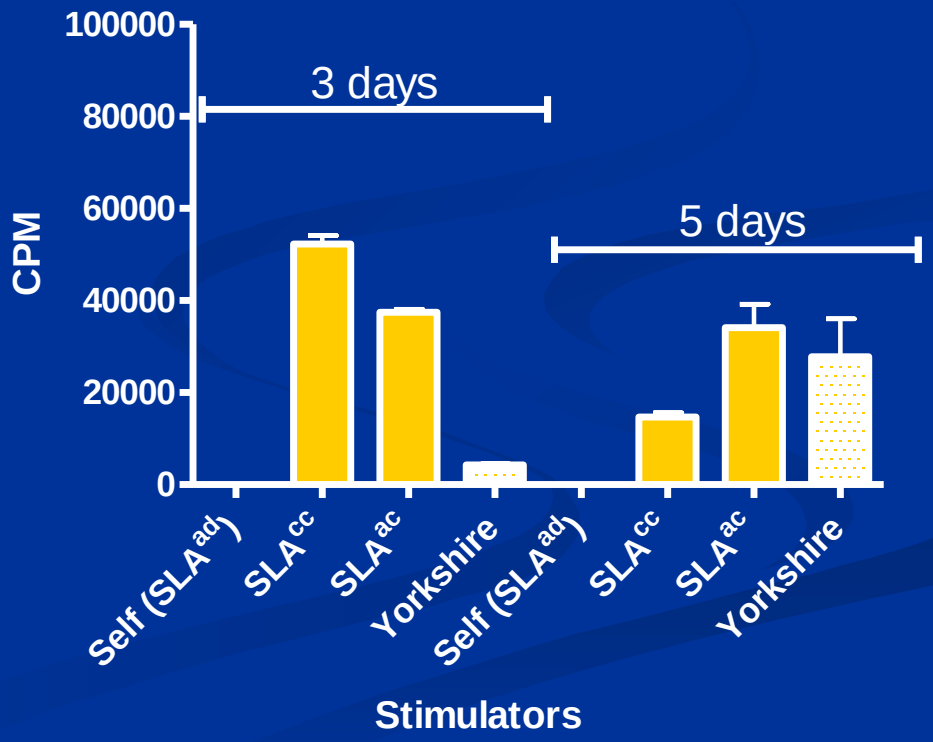
Cellular Responses

Mixed Lymphocyte Reactivity (MLR)

Naive SLA^{ad} Responders

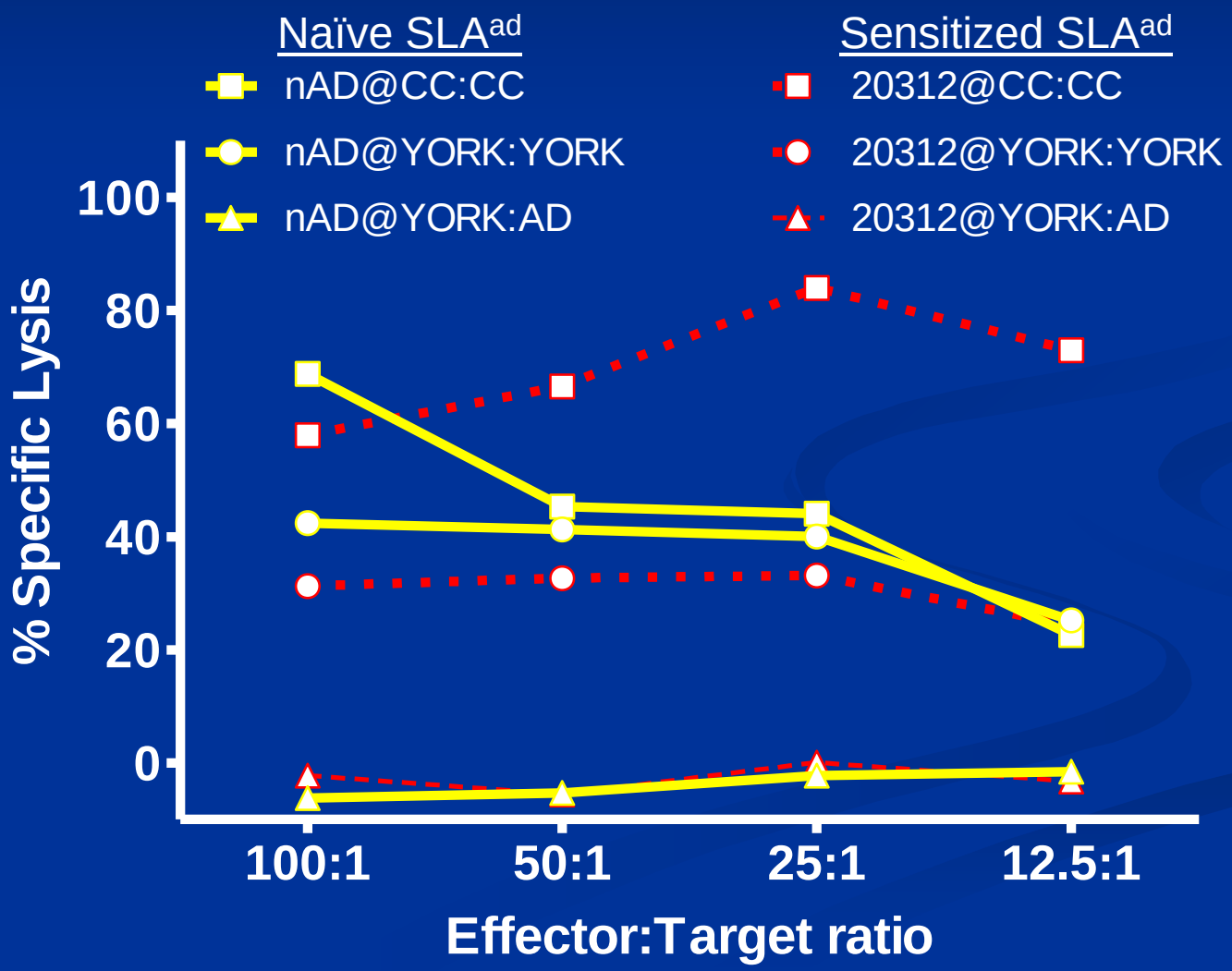


Sensitized SLA^{ad} Responders
(following SLA^{ac} skin graft rejection)



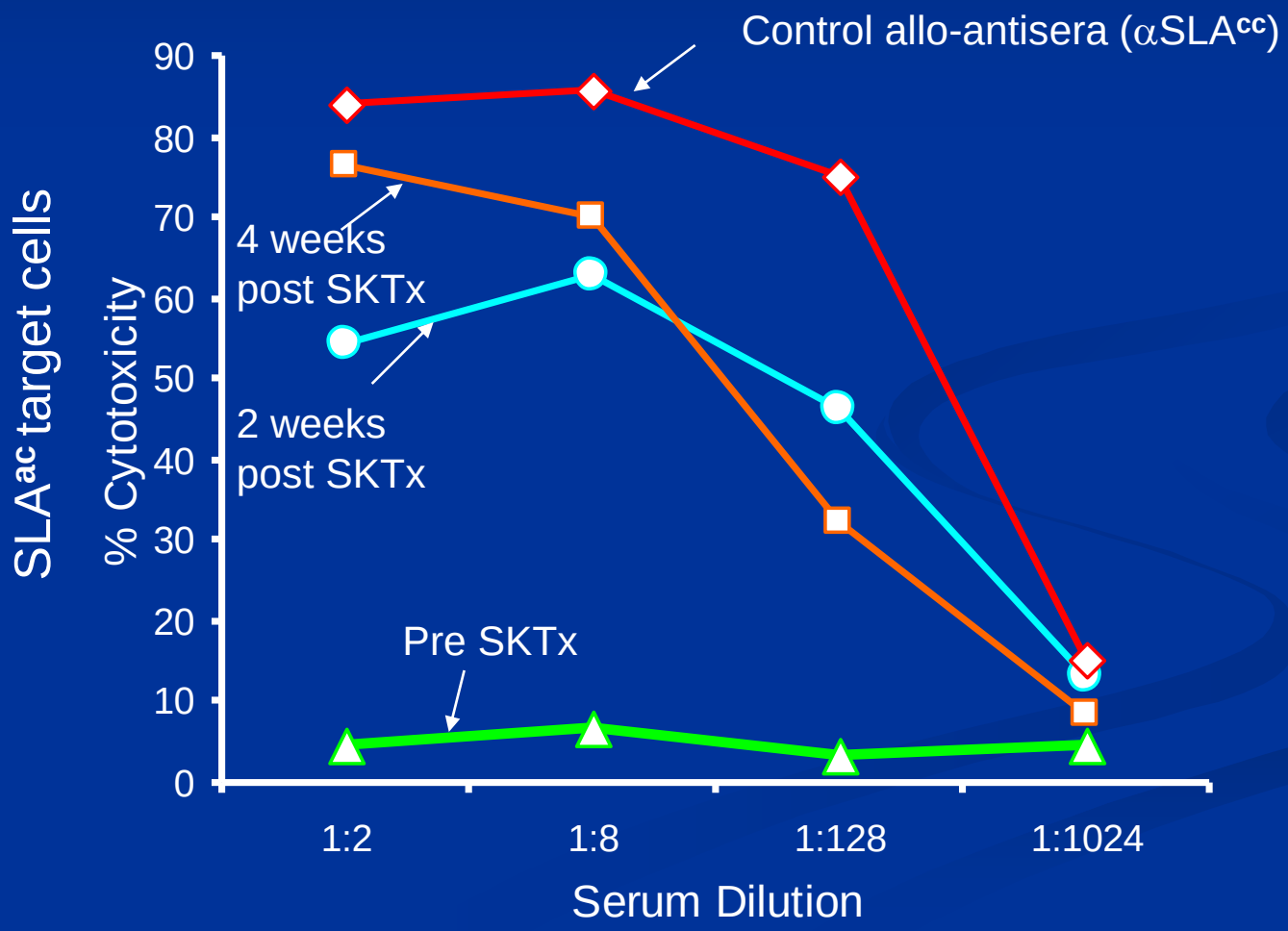
Cellular Responses

Cell Mediated Lympholysis (CML)



Antibody Responses

Sera from SLA^{ad} animal pre and post SLA^{ac} skin rejection



Hematopoietic cell transplantation and immune tolerance studies in miniature swine

(*Transplantation* 2006;81: 1677–1685)

Stable Multilineage Chimerism without Graft versus Host Disease Following Nonmyeloablative Haploidentical Hematopoietic Cell Transplantation

Robert A. Cina,[1,2] Krzysztof J. Wikiel,[1] Patricia W. Lee,[1,3] Andrew M. Cameron,[1,4] Shehan Hettiarachy,[1,5] Haley Rowland,[1] Jennifer Goodrich,[1] Christine Colby,[6] Thomas R. Spitzer,[6] David M. Neville, Jr.,[7] and Christene A. Huang[1,8]

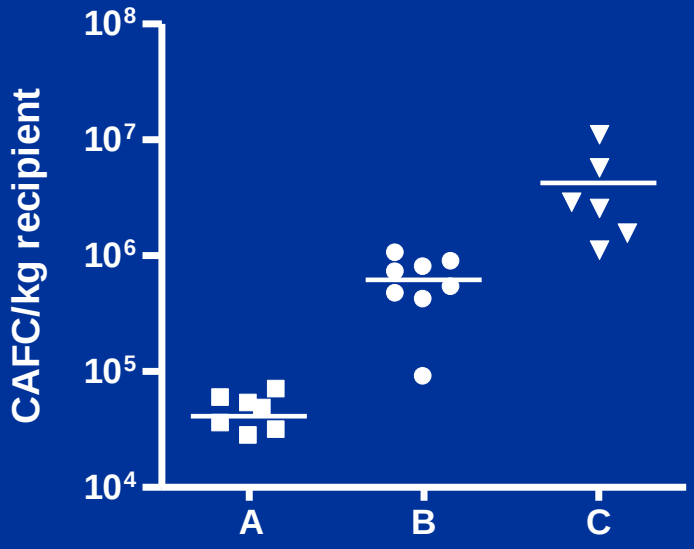
American Journal of Transplantation 2006; 6: 2894–2902
Blackwell Munksgaard

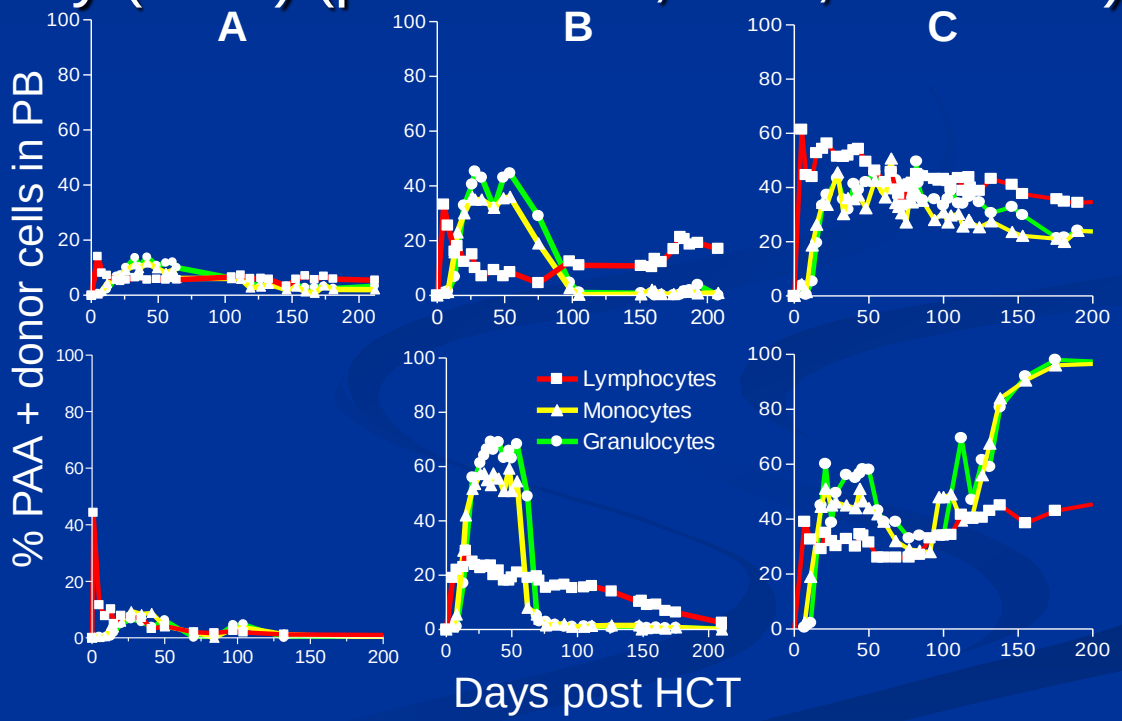
Predictors of Organ Allograft Tolerance Following Hematopoietic Cell Transplantation

B. M. Horner[a,b,*], R. A. Cina[a], K. J. Wikiel[a], B. Lima[a], A. Ghazi[a], D. P. Lo[a], K. Yamada[a], D. H. Sachs[a] and C. A. Huang[a]

Miniature Swine
Assessing hematopoietic stem cell function

- Cobblestone Area Forming Cell Assay (CAFC)
- Colony Forming Cell Assay (CFU) (porcine IL3, SCF, GM-CSF)





Amount of functional stem cell activity infused correlates with engraftment outcome

Tumor studies in miniature swine

(Blood. 2007;110: 3996-4004)

Establishment of transplantable porcine tumor cell lines derived from MHC-inbred miniature swine

Patricia S. Cho,[1,2] Diana P. Lo,[1] Krzysztof J. Wikiel,[1,2] Haley C. Rowland,[1] Rebecca C. Coburn,[1] Isabel M. McMorrow,[1,2] Jennifer G. Goodrich,[1] J. Scott Arn,[1] Robert A. Billiter,[1] Stuart L. Houser,[1] Akira Shimizu,[1] Yong-Guang Yang,[1,2] David H. Sachs,[1,2] and Christene A. Huang[1,2]

[1]Transplantation Biology Research Center, Massachusetts General Hospital (MGH), and [2]Harvard Medical School, Boston, MA

Veterinary Immunology and Immunopathology 135 (2010) 243–256

Myelogenous leukemia in adult inbred MHC-defined miniature swine: A model for human myeloid leukemias

Raimon Duran-Struuck [a,*], Patricia S. Cho [a], Alexander G.S. Teague [a], Brian Fishman [a], Aaron S. Fishman [a], John S. Hanekamp [a], Shannon G. Moran [a], Krzysztof J. Wikiel [a], Kelly K. Ferguson [a], Diana P. Lo [a], Michael Duggan [a], J. Scott Arn [a], Bob Billiter [a], Ben Horner [a], Stuart Houser [a], Beow Yong Yeap [b], Susan V. Westmoreland [c], Thomas R. Spitzer [d], Isabel M. McMorrow [a], David H. Sachs [a], Roderick T. Bronson [e], Christene A. Huang [a]

[a] *Transplantation Biology Research Center, Massachusetts General Hospital, United States*
[b] *Biostatistics Center, Massachusetts General Hospital, United States*
[c] *New England Primate Research Center, Harvard Medical School, United States*
[d] *Cancer Center, Massachusetts General Hospital, United States*
[e] *Histopathology Core, Harvard Medical School, United States*

Limitations of Large Animal Models

- Expense
  - Need for large animal facility
    - OR capability
  - Limited animal numbers per group
- Cannot assess immune response of human cells/constructs directly
  - Xeno-responses differ from allo-responses
  - Need to generate analog of human cells

MGH Miniature Swine

- The MGH Miniature Swine Colony, developed in the mid-70s by David H. Sachs, MD, originated from a cross of two separate miniature swine lineages (**Fig 1**). The colony moved to MGH from NIH in 1990 and have been used extensively by many investigators for immunologic, physiologic and imaging studies.
- In 2007, a 3 million-dollar renovation of the Swine 2 Research facility, located on the Tufts Cummings School of Veterinary Medicine campus, was completed. This state-of-the-art facility , funded by NCRR, includes multiple farrowing and nursery rooms, OR, wetlabs and AI lab (**Fig 2**), as well as the main housing area (**Fig 3**).
- The MGH Miniature Colony is a closed colony for biosecurity reasons and once animals leave the facilty, they may not re-enter. Animal care personnel are required to shower within the facility prior to entry and the operation conforms to the USDA Research guidelines. Figure 4 shows a complete growth profile of the herd.
- While the primary focus of this herd is to facilitate preclinical immunologic studies ongoing at Massachusetts General Hospital, surplus animals are available to area institutions for scientific research purposes. **Appendix 1** outlines the routine herd health program, while **Appendix 2** details the biosurveillance program.

- ***Please call (617)298 0511 for further inquiries***

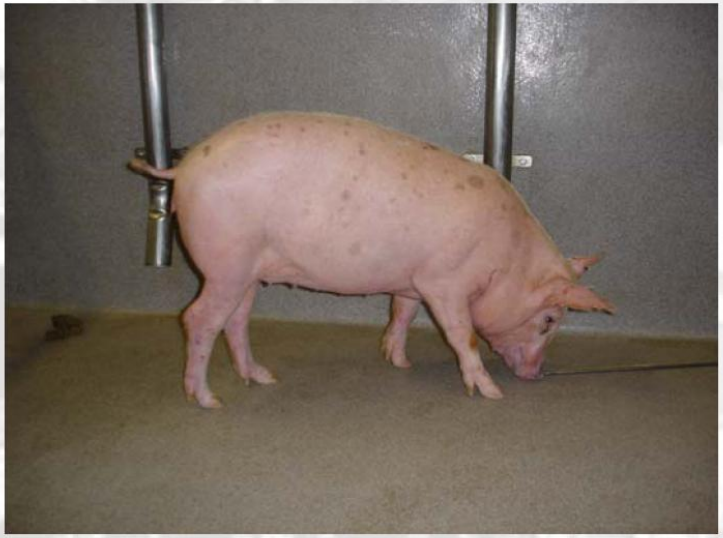


Figure 1

Main housing area

A.I. Laboratory

- **MGH Miniature Swine**

This herd is located in the state of Massachusetts which is USDA-recognized Brucellosis and Pseudorabies (Aujeszky's Disease) free. In addition, quarterly testing confirms that this colony is free of Transmissable Gastroenteritis (Elisa) and Porcin Reproductive and Respiratory Syndrome (Elisa).

Vaccination: All piglets are vaccinated against Mycoplasma Hyopneumoniae, Hemophilus Parasuis, Streptococcus Suis, Pasteurella Multocida, Bordatella Bronchiseptica and Erysipelothrix Rhusiopathiae at day 7, with a booster vaccination at 28 days of age. All swine six months or older are vaccinated against Erysipelothrix Rhusiopathiae, Leptospira (Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona), Influenza and Parvovirus. Repeat vaccination is performed every six months.

Parasite Control: A herdwide deworming program with ivermectin is performed every six months.

• MGH Miniature swine

- **Biosecurity/Biosurveillance:** Quarterly bleeds are performed on 10-15% of the breeding animals and submitted to Iowa State Veterinary Diagnostic Laboratory for a complete serology panel (see below). In addition, quarterly nasal and rectal swabs cultures are performed. All animal care personnel are required to shower prior to entering the facility.

Organism or Disease	Iowa State Testing Method	
Encephalomyocarditis Virus	VN	
Leptospira	MAT	
Mycoplasma hyopneumoniae	CF or ELISA	
Porcine Respiratory & Reproductive Syndrome	ELISA or IFA	
Porcine Parvovirus	HI	
Pseudorabies	G1 ELISA VN ELISA Screen ALA	
Swine Influenza Virus	HI (H1N1 and H3N2) ELISA (H1N1 only) PFIZER (H1N1 only)	
Transmissible Gastroenteritis Virus	VN	
Vesicular Stomatitis Virus (Indiana) Vesicular Stomatitis Virus (New Jersey)	VN VN	
Bovine Viral Diarrhea type 1 Bovine Viral Diarrhea type 2	VN VN	
Brucellosis	Card test (B. abortus/suis)	
Toxoplasmosis	LAT	

CF – Complement Fixation
ELISA – Enzyme-Linked Immunosorbent Assay
HI – Hemagglutination inhibition
LAT – Latex agglutination
MAT – Microscopic agglutination test
VN – Virus Neutralization