

Humanized Mice for Studies of Infectious Diseases

A Scientific Framework for Epidemic and Pandemic Research Preparedness

January 9, 2024

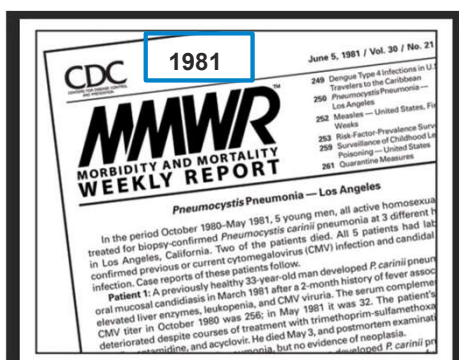
Professor Lenny Shultz
The Jackson Laboratory

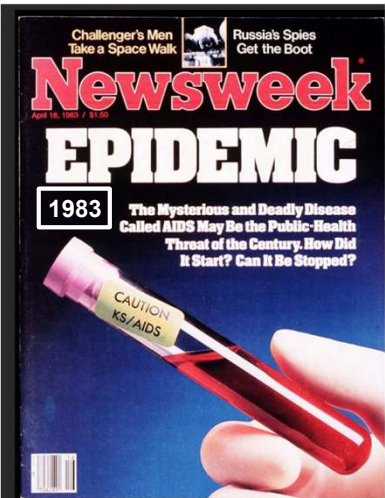





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Animal Models Were Critically Needed to Test HIV Pathogenesis and Therapeutics





Since 1980 ~ 85 million people have been infected with HIV

2022, ~39 million people are living with HIV
An estimated > 50 million deaths

UN AIDS Facts Sheet

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Humanized CB17-scid Mice Engrafted With Fetal Human Tissues or PBMC Support HIV Infection

Infection of the SCID-hu Mouse by HIV-1

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SCID-hu mice with human fetal thymic or lymph node implants were inoculated with the cloned human immunodeficiency virus-1 isolate, HIV-1_{JR-CSF}. In a time- and dose-dependent fashion, viral replication spread within the human lymphoid organs. Combination immunohistochemistry and in situ hybridization revealed only viral RNA transcripts in most infected cells, but some cells had both detectable viral transcripts and viral protein. Infected cells were always more apparent in the medulla than in the cortex of the thymus. These studies demonstrate that an acute infection of human lymphoid organs with HIV-1 can be followed in the SCID-hu mouse.

Science (1988) 242:1684

Human Immunodeficiency Virus Infection of Human-PBL-SCID Mice

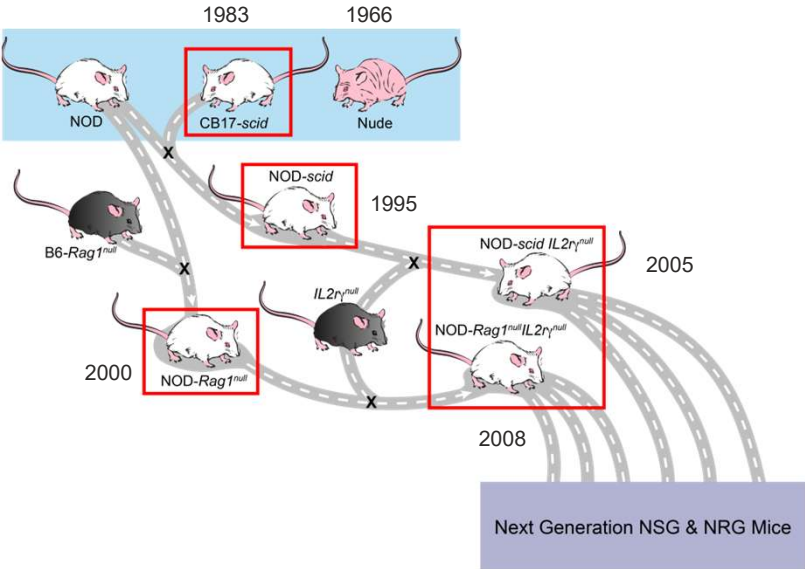
DONALD E. MOSIER, RICHARD J. GULIZIA, STEPHEN M. BAIRD, DARCY B. WILSON,* DEBORAH H. SPECTOR, STEPHEN A. SPECTOR

Severe combined immunodeficient (SCID) mice reconstituted with human peripheral blood leukocytes (hu-PBL-SCID mice) have inducible human immune function and may be useful as a small animal model for acquired immunodeficiency syndrome (AIDS) research. Hu-PBL-SCID mice infected with human immunodeficiency virus-1 (HIV-1) contained virus that was recoverable by culture from the peritoneal cavity, spleen, peripheral blood, and lymph nodes for up to 16 weeks after infection; viral sequences were also detected by in situ hybridization and by amplification with the polymerase chain reaction (PCR). Mice could be infected with multiple strains of HIV-1, including LAV-1/Bru, IIIB, MN, SF2, and SF13. HIV-1 infection affected the concentration of human immunoglobulin and the number of CD4⁺ T cells in the mice. These results support the use of the hu-PBL-SCID mouse for studies of the pathogenesis and treatment of AIDS.

Science (1991) 251:791

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The Road to Humanized Mice



Shultz et al (2007) Nat Rev Immunol 7:118

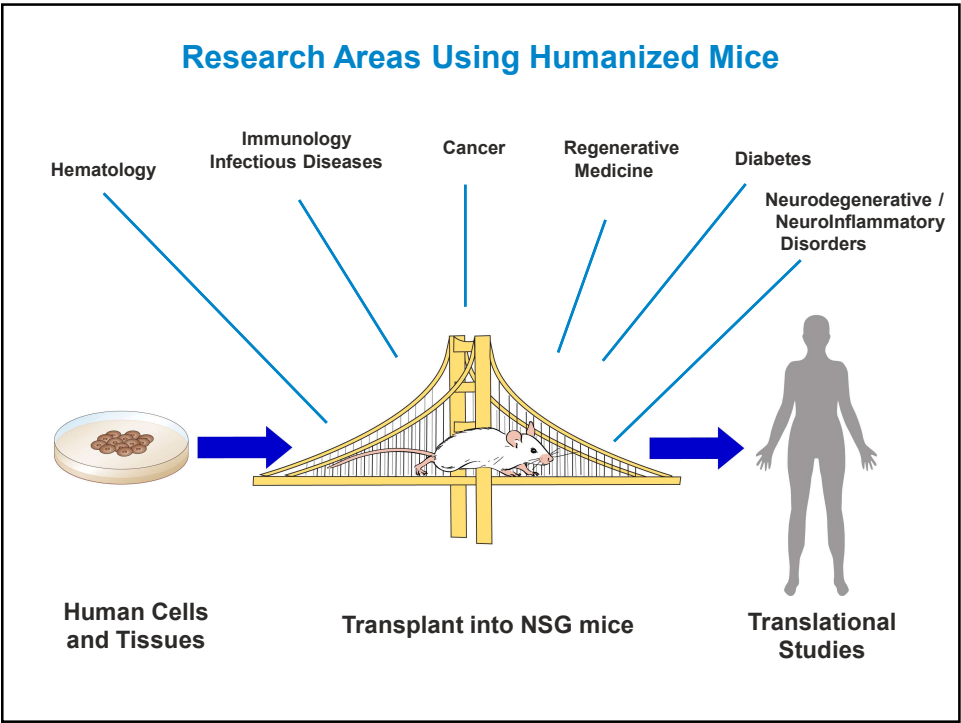
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Major Humanized Mouse Strain Platforms

NSG	NOD- <i>scid</i> <i>IL2rg</i> ^{null}	Jackson Laboratory
NRG	NOD- <i>Rag1</i> ^{null} <i>IL2rg</i> ^{null}	Jackson Laboratory
NOG	NOD- <i>scid</i> <i>IL2rg</i> ^{Trunc}	CIEA (Tokyo)
NCG	NOD- <i>scid</i> <i>IL2rg</i> ^{null}	Charles River
NSI	NOD-TALEN- <i>scid</i> <i>IL2rg</i>	South China Inst Stem Cell
BRG	BALB/c- <i>Rag2</i> ^{null} <i>IL2rg</i> ^{null}	Yale/Univ. Hosp. Zurich
BRGS	BALB/c- <i>Rag2</i> ^{null} <i>IL2rg</i> ^{null} <i>NOD</i> ^{SIRPa}	Univ. of Amsterdam
B6	B57BL/6- <i>Rag2</i> ^{null} <i>IL2rg</i> ^{null} <i>CD47</i> ^{null}	NIAID/Stanford Univ.
Many specialized strains with limited availability ie MISTRG		

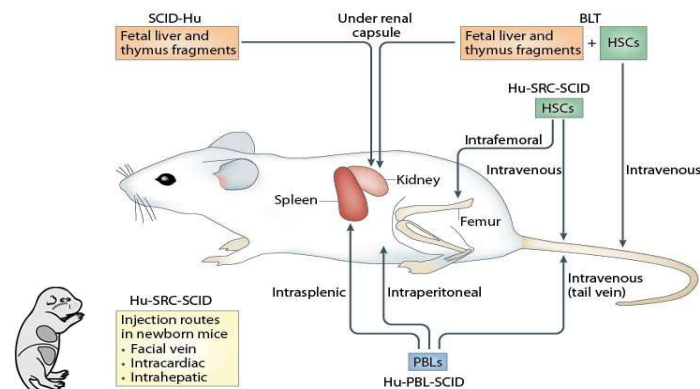
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Research Areas Using Humanized Mice



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Engraftment of NSG Mice With Human Hematopoietic Cells and Tissues



LD Shultz et al (2012) Nat Rev Immunol 12:786

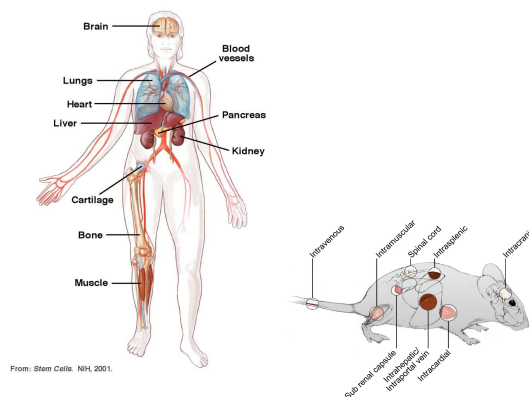
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Humanized Mice Play a Major Role in Regenerative Medicine

Human Stem Cells

ES cells
iPS cells

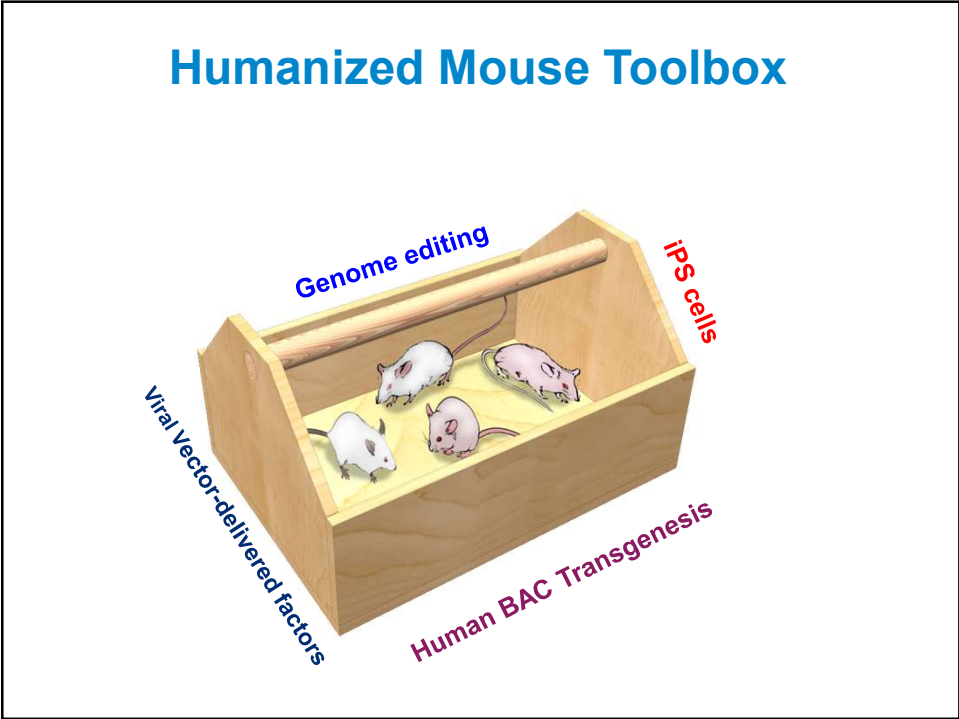
Hematopoietic
Mesenchymal
Neural
Hepatic
Pancreatic beta cells
Skeletal muscle
Cardiac muscle
Skin epithelium
Lung epithelium
Intestinal epithelium



Collaborations on projects focused on human stem cell-derived populations

Rudy Jaenisch - **Microglia**
Doug Melton - **Pancreatic beta cells**
George Daley - **Hematopoietic stem cells**
Rene Meier - **Thymic epithelial cells**
Charles Emerson - **Skeletal muscle stem cells**

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Next Generation Humanized Mice

Transgenic expression of human factors

- Cytokines
- HLA molecules
- Microenvironmental factors
- Hormones

Reduced mouse innate immunity & HSC function

- MHC class I and II
- Cytokines
- Macrophages
- Granulocytes
- Dendritic Cells
- Chemokine receptors
- Interferon receptors
- Toll-like receptors
- c-Kit receptor
- Thymus

Increased Innate Immunity

- Restore hemolytic complement

Mouse stromal marker

- Ubiquitous GFP expression

Disease Models

NOD ES cells validated for germ-line transmission and used to target genes in NSG mice

Ongoing studies use site directed nucleases: TALENS, CRISPR/Cas9

Human BACs used to provide transgenes with endogenous promoters

NOD-*scid*

IL2r^{null}

X

NOD-*scid* *IL2r^{null}*

NSG

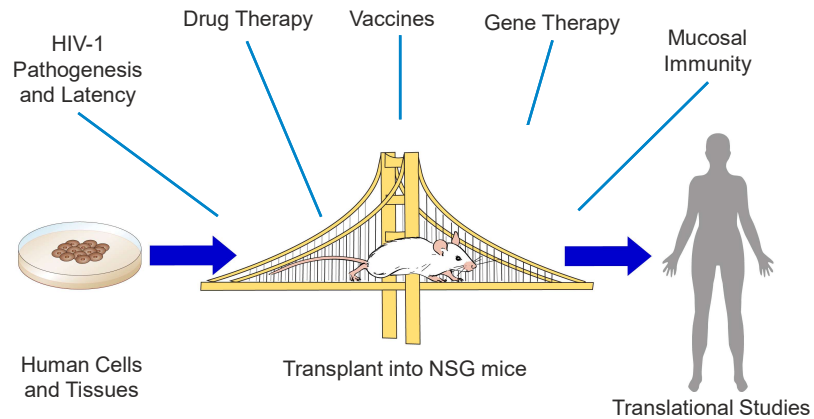
NSG-*Foxn1^{nu}*

NSG-Tg(CAG-eGFP)

L Shultz et al (2012) Nat Rev Immunol 12:786

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HUMANIZED MICE FOR HIV RESEARCH

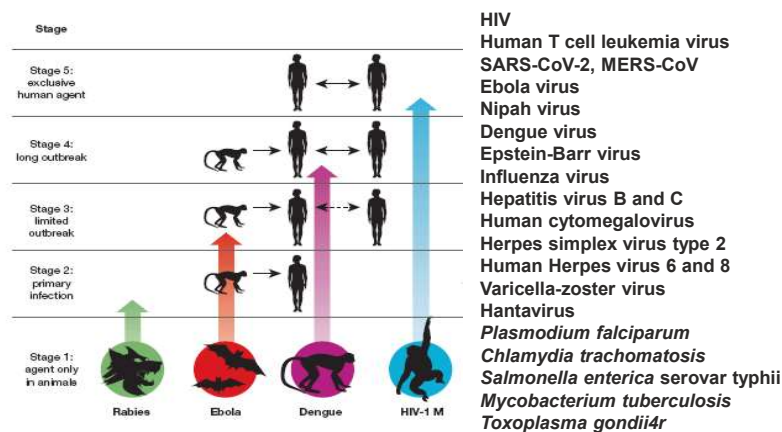


Improvements and Limitations of Humanized Mouse Models for HIV Research:
NIH/NIAD "Meet the Experts" 2015 Workshop Summary (18 Labs represented)

R Akkina et al (2016) AIDS Research Human Retroviruses 32:109

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Human Infectious Agents Studied in Humanized Mice

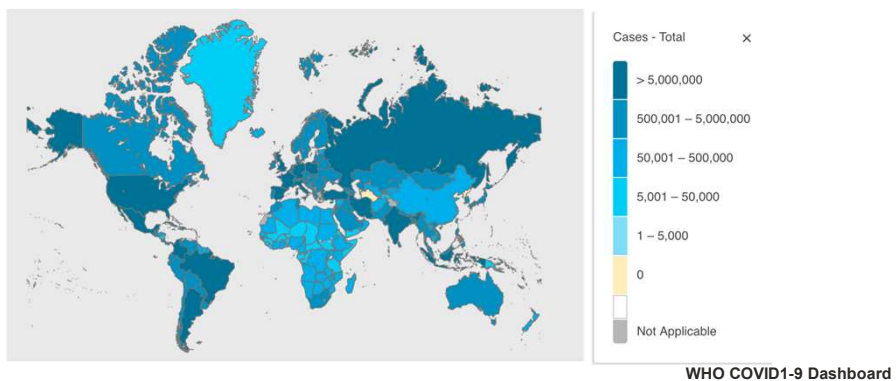


N Wolfe et al (2007) Nature 447:279
M Brehm et al (2013) Curr Opin Immunol 25:428
M Brehm et al (2014) J Immunol Methods 410:3
Walsh NC et al (2017) Annu Rev Pathol 12:187
Valbuena, G et al (2023) Methods Mol Biol 2682:191

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COVID Cumulative Cases and Deaths

Region	Cumulative cases	Cumulative deaths
Global	773 million	6.9 million
USA	103 million	1.1 million
China	> 100 million	~ 1.5 million



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Humanized Mouse COVID Models

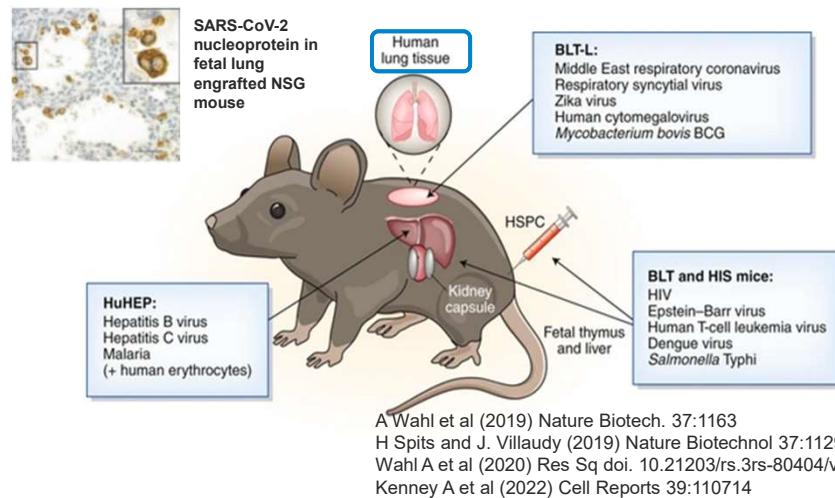
Infection of engrafted fetal lung (NSG)

Transient expression of ACE-2 in lungs by Adeno-associated virus (MISTRG6)

Transgenic expression of ACE-2 (NSG)

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Engraftment of Fetal Human Tissue in NSG Mice Supports Infection with SARS-CoV-2, MERS-CoV, Zika Virus, Dengue Virus, Nipah Virus and Other Human Pathogens



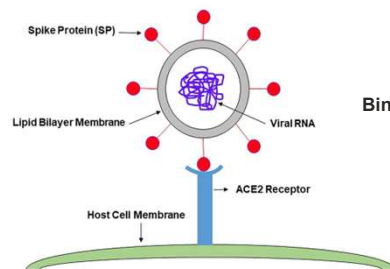
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NSG-Tg(Hu-ACE2) Mice Under Study

NSG-Tg(K18-Hu-ACE2) Keratin 18 promoter, random integration and copy number

NSG-Tg ROSA26 (K18Hu-ACE2) Keratin 18 promoter, integrated into ROSA26 locus; single copy

NSG-(Ace2^{tm1ACE2}) Human ACE2 gene replacing endogenous mouse Ace2 sequences. Expression directed by human regulatory elements

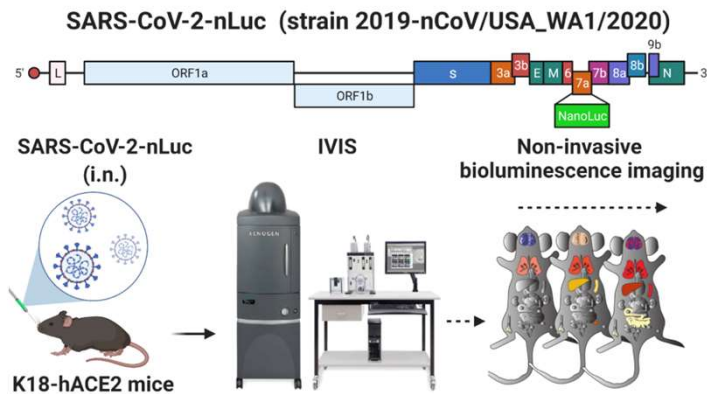


Binding of SARS-CoV-2 spike protein with human ACE2

M Singh et al (2022) Mol Cell Biochem 22:1

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Bioluminescence Imaging of SARS-CoV-2 Replication Dynamics in B6 K18-Hu-ACE2 Transgenic Mice



SARS-CoV-2 carrying nLuc reporter in ORF7a for non-invasive BLI spread of virus following intranasal (i.n.) challenge
ORF7a deletion does not affect infectivity or pathology. Provides real-time daily readout of progressing infection.

Ullah H et al (2021) Immunity 54:2143

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Summary

Humanized mice support engraftment of multiple hematopoietic as well as non-hematopoietic human cells and tissues

Infection of the engrafted human cells allow mechanistic and therapeutic studies of infectious diseases without putting patients at risk

Expression of ACE-2 receptor in NSG mice supports therapeutic studies of SARS-CoV-2 variants

Advances in genomic editing of humanized mice and in human iPS cell technology will support rapid advances in the application of humanized mice to epidemic and pandemic research

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Acknowledgements

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Stanford University Joseph Wu Nigel Kooreman RIKEN Res Ctr Fumihiko Ishikawa Yoriko Saito	SUNY at Buffalo Richard Bankert Steven Bernstein Kunle Odunsi Harvard Med School Doug Melton		