Islet Transplantation:
from bench to bedside

What is diabetes?

- Diabetes is a disease in which your body cannot properly store and use fuel for energy.
- The fuel that your body needs is called glucose. Glucose comes from foods such as breads, cereals, pasta, rice, potatoes, fruits and some vegetables.
- To use glucose, your body needs insulin. Insulin is made by a gland in your body called the pancreas.

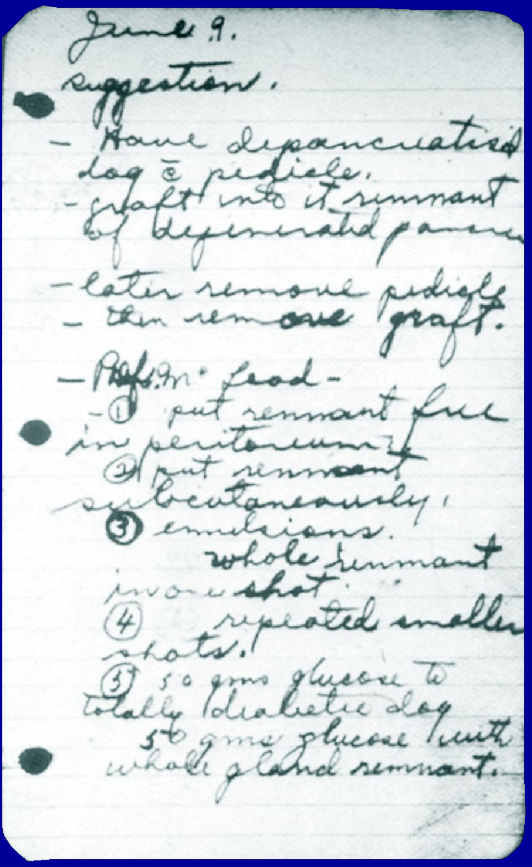
(www.diabetes.ca)

The prevalence of diabetes

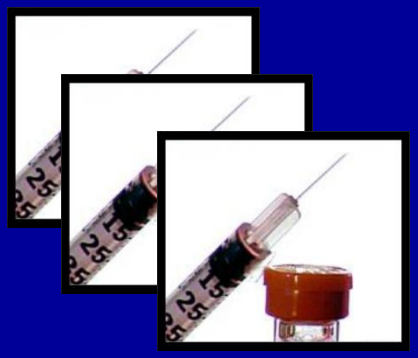
- In 2000, the World Health Organization estimated that over 177 million people have diabetes.
- By 2025, this figure will top 300 million.
- More than 2 million Canadians have diabetes. By the end of the decade, this number is expected to rise to 3 million.
- Approximately 10% of people with diabetes have type 1 diabetes. However, the number of people with type 2 diabetes is increasing dramatically.

(www.diabetes.ca)

Discovery of Insulin
(1921-1922)


June 9.
Suggestions.
- Have depancreatised dog & pedicle.
- graft into it remnant of depancreated pancreas
- later remove pedicle
- then remove graft.
- Proposed - Head -
(1) put remnant free in peritoneum
(2) put remnant subcutaneously
(3) emulsions. whole remnant in one shot.
(4) repeated smaller shots.
(5) 50 gms glucose to totally diabetic dog
50 gms glucose with whole gland remnant.

! Insulin prevented acute death



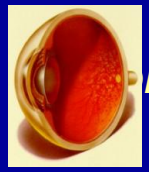
Insulin Therapy – Problems

Possible side effects include

- **…od sug… …nia)**
- **…ction**
- **allergic reactions**

30% of patients with Type 1 Diabetes die of kidney failure

…ing ar… Leading cause of death of diabetics



…r prol… …ons

Retinopathy is leading cause of new blindness

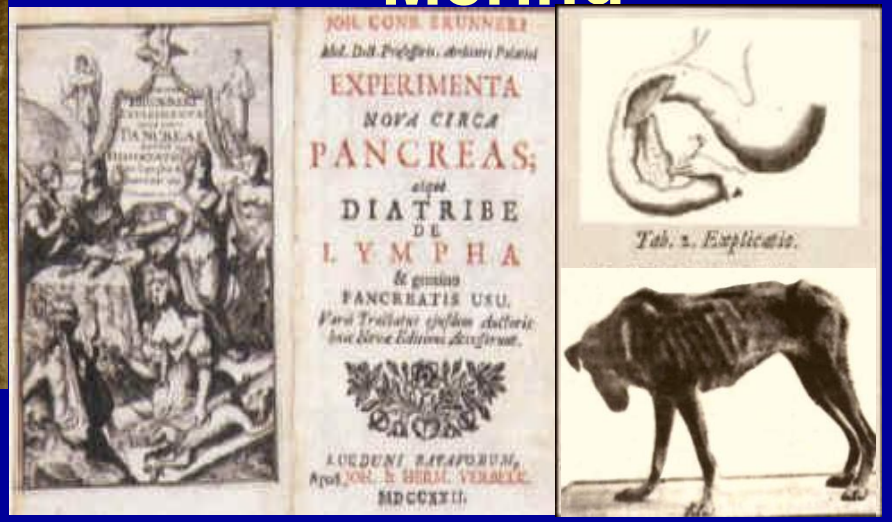
Islet Transplantation:

an alternative treatment for type 1 diabetes

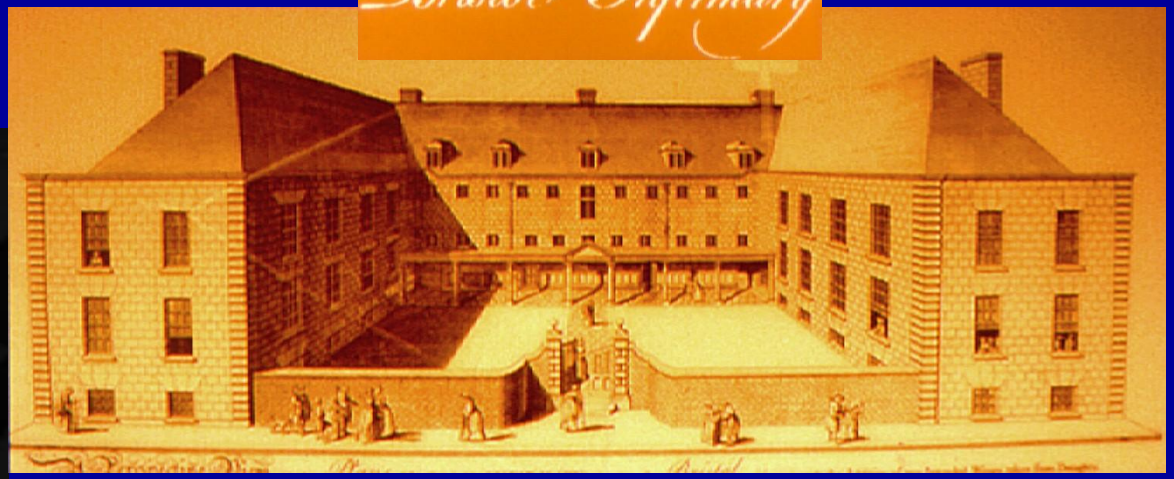
Oscar Minkowski Joseph Von Mering

Joseph Von Mering (1849 - 1908)

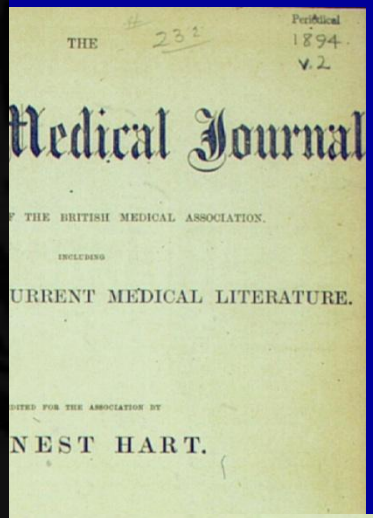
Oscar Minkowski (1858 - 1931)



Surgical removal of pancreas caused glycosuria



189
3


THE

Medical Journal

THE BRITISH MEDICAL ASSOCIATION.

INCLUDING

URRENT MEDICAL LITERATURE.

NEST HART.

VOLUME II FOR 1894.

JULY TO DECEMBER.

NOTES ON DIABETES TREATED WITH EXTRACT AND BY GRAFTS OF SHEEP'S PANCREAS.

By P. WATSON WILLIAMS, M.D.Lond.,

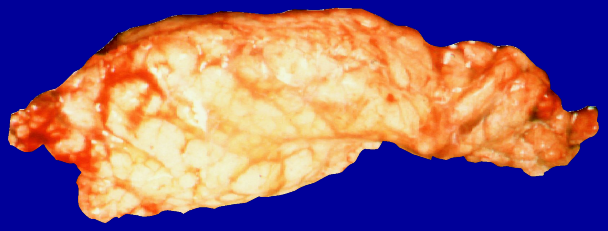
Senior Assistant Physician Bristol Royal Infirmary.

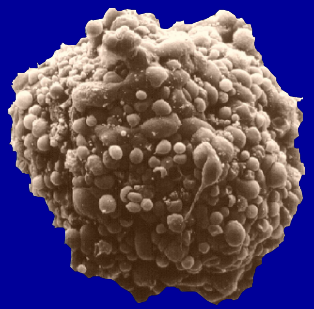
Islet Isolation

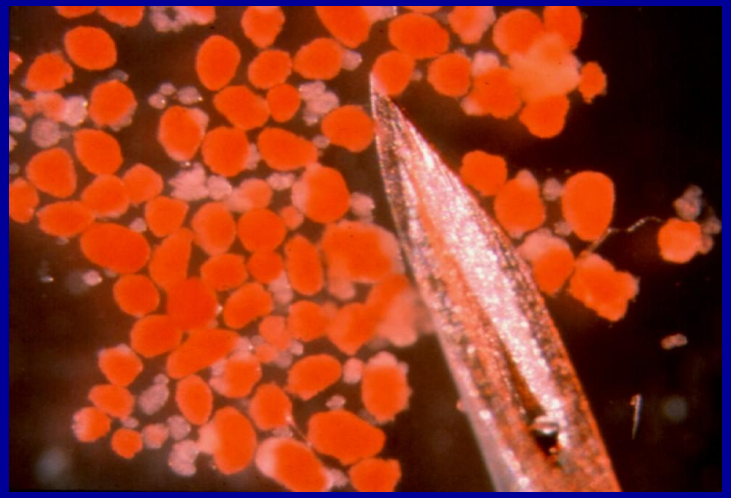
1904 – The idea was presented by Ssobolev

1911 – Bensley handpicked islets from rodents

1964 – Hellerstrom described the first method of islet isolation by hand dissection under the microscope





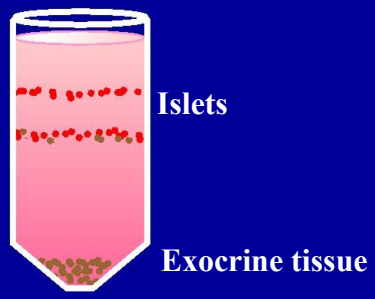


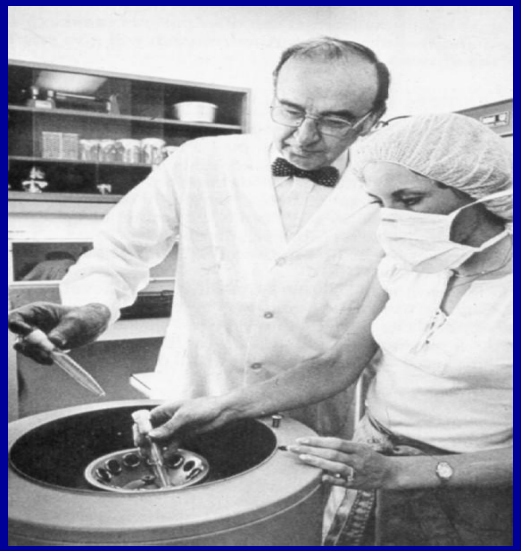
Major Contributions to Islet Isolation and Transplantation

1964 – Moskalewski used collagenase digestion of islets in guinea pigs

1965 – Lacy and Kostianovsky, retrograde perfusion via common bile duct

1967 – Lacy and Kostianovsky used sucrose density gradient – 300 islets/rat pancreas





Major Contributions to Islet Isolation and Transplantation

1969 – Lindall et al. used Ficoll density gradient

1970 – Younoszai et al., first report on transplanting isolated rat islets, effect was temporary

1972 – Ballinger and Lacy demonstrated a sustained and significant reduction of blood glucose by transplanting 400 - 600 islets i.p. or i.m.

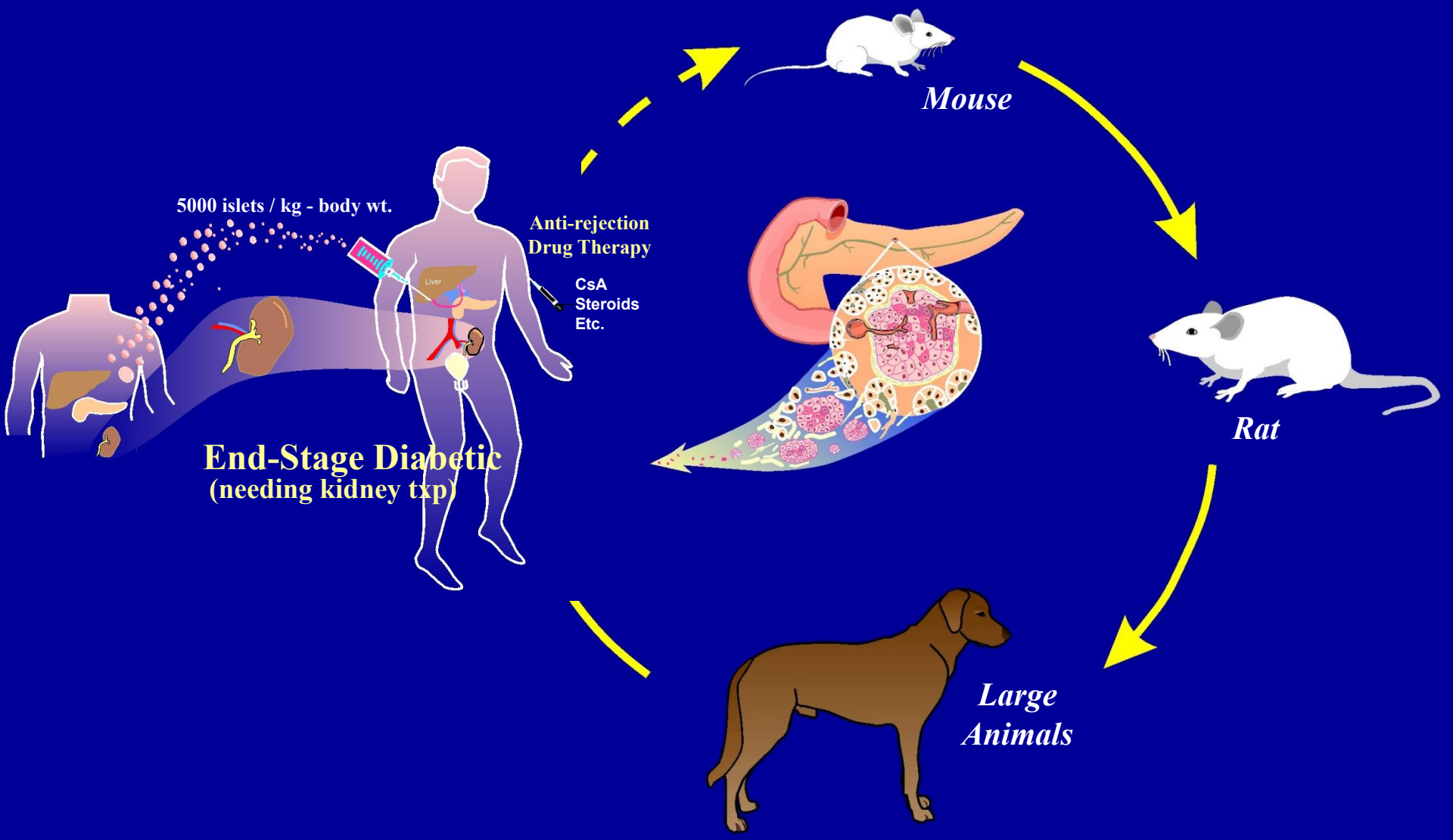
Major Contributions to Islet Isolation and Transplantation

1973 – Reckard, Ziegler and Barker demonstrated complete normalization of blood glucose in rats for as long as 7 months using 800 - 1200 islets i.p.

1973 – Kemp et al., demonstrated that approximately 800 islets transplanted into the liver completely normalize blood glucose levels in rats

Bench to Bedside

1972 – 1989



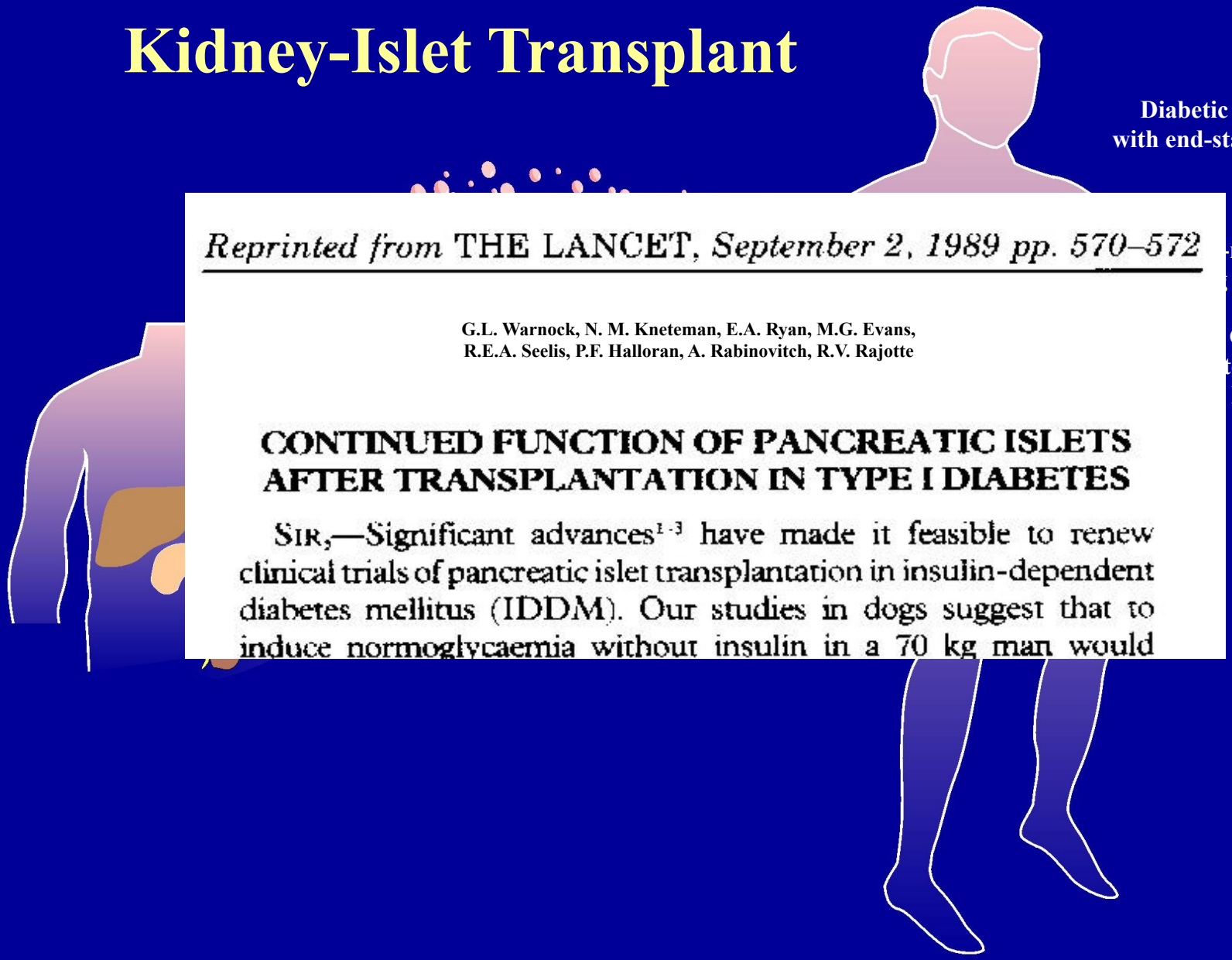
Edmonton
Minneapolis
Pittsburgh
St. Louis
Miami
Leicester
Oxford
Paris
Perugia
Milan
1989
Islet TRANSPLANT
WORLD WIDE

Combined Kidney-Islet Transplant

Diabetic patients
with end-stage disease

-rejection
Therapy

CsA
teroids
Etc.



Reprinted from THE LANCET, *September 2, 1989 pp. 570–572*

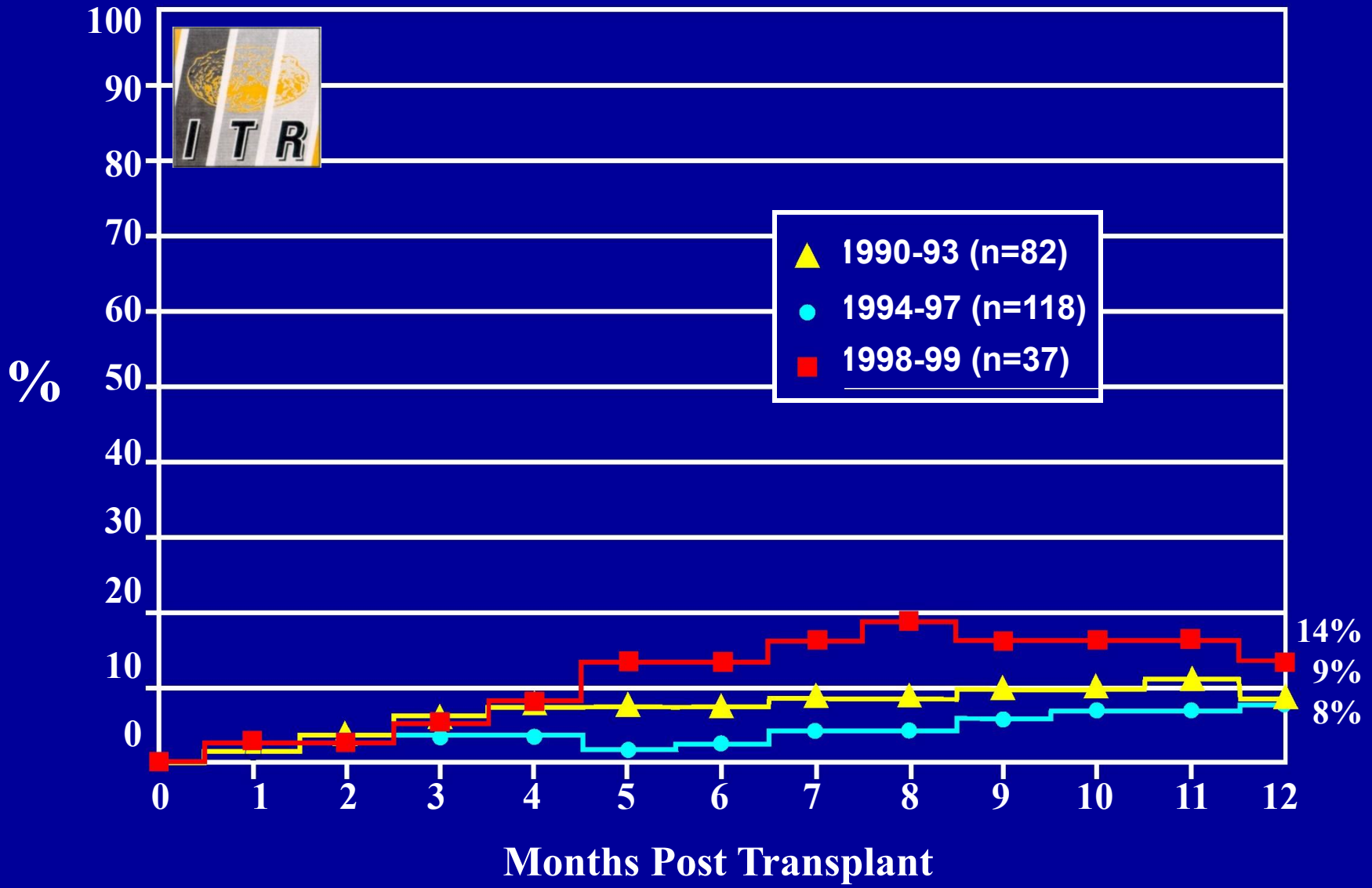
G.L. Warnock, N. M. Kneteman, E.A. Ryan, M.G. Evans,
R.E.A. Seelis, P.F. Halloran, A. Rabinovitch, R.V. Rajotte

CONTINUED FUNCTION OF PANCREATIC ISLETS AFTER TRANSPLANTATION IN TYPE I DIABETES

SIR,—Significant advances[1-3] have made it feasible to renew clinical trials of pancreatic islet transplantation in insulin-dependent diabetes mellitus (IDDM). Our studies in dogs suggest that to induce normoglycaemia without insulin in a 70 kg man would

Graft Survival (Insulin Independence)

Islet Transplant Registry



Only well documented cases

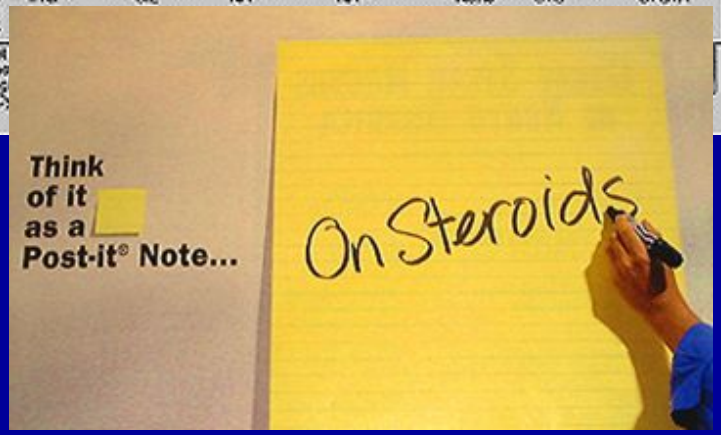
Drugs

- Steroid
- Cyclosporine
- Azathioprine

Type I Diabetic Recipients of Adult Islet Allografts
Summary of Insulin-Independent Cases (n=27) through Dec 31, 1995 (2)

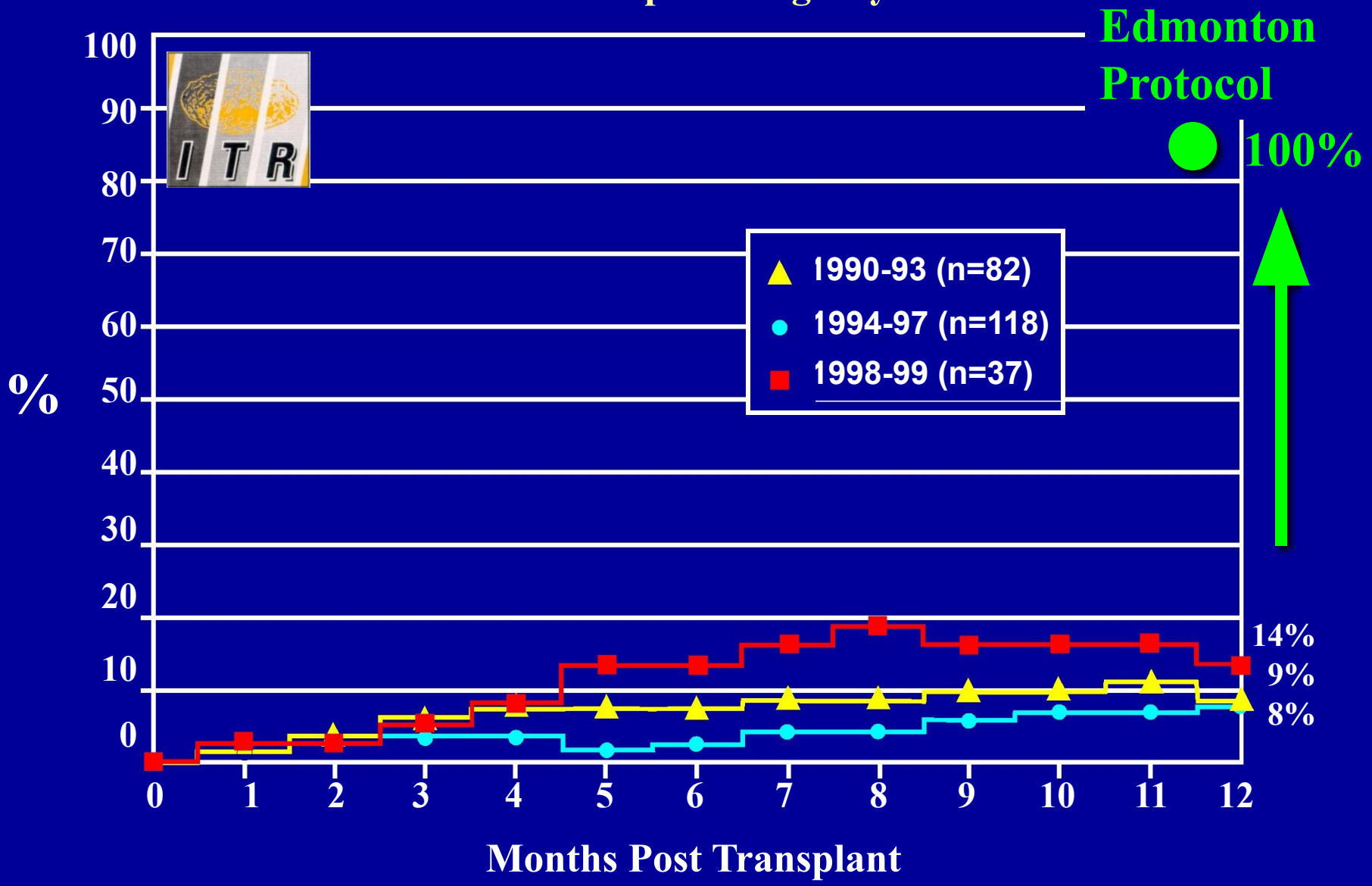
Institution¹	Year of Tx	Site of Tx	Recipient Category	# of Shared HLA-Ag A/B	# of Shared HLA-Ag DR	Immunosuppression Induction	Immunosuppression Maintenance
Zurich	1978	spleen	SIK	1	0	ALG +S+A+CPM	S+A
Paris	1988	epiploic flap	SIL	1	1	ALG +S+C+A	S+C+A
St. Louis	1989	liver	IAK	1/3	2/1	ALG +S(SD)+C+A	C+A
St. Louis	1990	liver	IAK	1/3?2	1/1/0	ALG +S+A	S+A
St. Louis	1993	liver	SIK	1/0/1/1/0/0/1/0	1/0/1/0/1/1/0/1	OKT3 +S+C+A	S+C+A
St. Louis	1993	liver	SIK	1/1/2/1/1	1/1/1/2/1	ATG +S+C+A	S+C+A
monton	1990	liver	SIK	3/1/3/1/0	0/0/0/0/0	ALG +S+A	S+C+A
monton	1992	liver	SIK	3/1/3/3/1/0	1/1/1/0/0/1	ALG +S+A	S+C+A
an	1990	liver	IAK	1	0	ALG +S+C+A	S+C+A
an	1990	liver	IAK	1/2	1/0	ALG +S+C+A	S+C+A
ah	1991	liver	SIK	NA	NA	ALG +S+C+A	S+C+A
ah	1992	liver	IAK	NA	NA	ALG +S+C+A	S+C+A
an	1994	liver	IAK	0/1/2	NA	ATG +S+C+A	C+A
an	1995	liver	IAK	0	NA	ATG +S+C+A	S+C+A
nni	1990	liver	IAK	0/3/3	1/1/0	OKT3 +S+C+A	S+C+A
nni	1990	liver	IAK	0/0/3	0/1/0	OKT3 +S+C+A	S+C+A
nni	1995	liver	SIK	0	0	ATG +S+THMMF	S+THMMF
nnapolis	1992	liver	SIK	1	1	ALG +S+C+A+D	S+C+A
nnapolis	1992	liver	SIK	2	0	ALG +S+C+A+D	S+C+A
nnapolis	1995	liver	IPP	2	1	None +S+C+MMF	S+C+MMF
ssen	1992	liver	IAK	2	1	ATG +S+C	S+C
ssen	1995	liver	ITA	1	0	anti-CD4 +S(SD)+C	-
ssen	1995	liver	SIK	1	1	ATG +S+C+A	S+C+A
ssen	1995	liver	SIK	2	1	ATG +S+C+A	S+C+A
ppa/Verona	1993	liver	SIL	NA	NA	None S+C	S+C+A
tsburgh	1994						
ensoMilan	1995						

et Transplant/ Islet
Simult. Islet Kidne
teroids SD Sing
acrolimus CPM Cy



Graft Survival (Insulin Independence)

Islet Transplant Registry



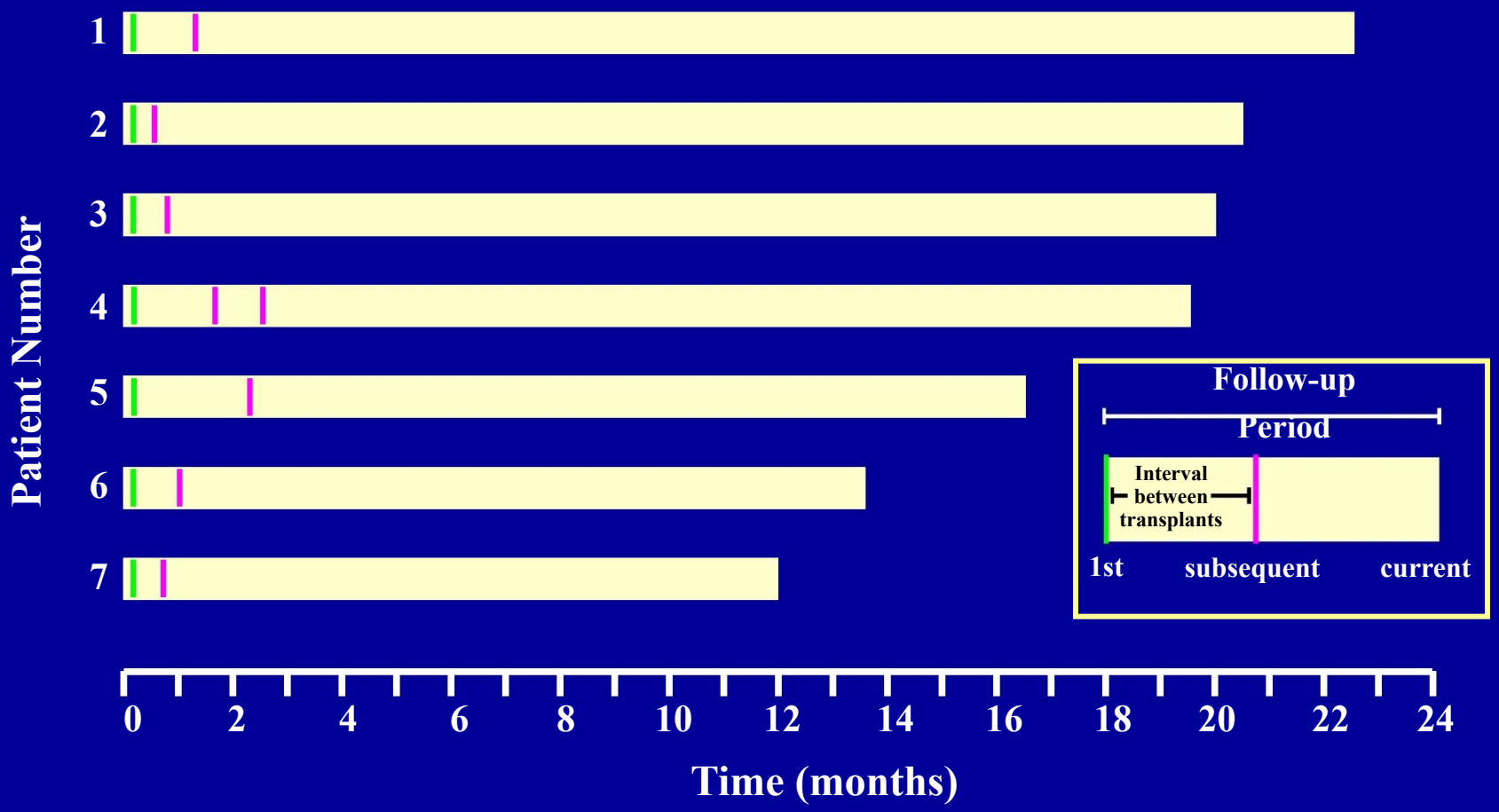
Only well documented cases

- Improved islet isolation
  - Liberase - Controlled perfusion
  - Gentle mechanical dissociation - No xenoproteins
- Immediate transplantation - No culture
- Transplant mass >10,000 IE/Kg

"Brittle" Diabetic Patients – Islet Transplant

Anti-rejection Therapy – No Steroids

Induction RX (10 wks) Zenopax (Daclizumab)
(Humanized Anti IL-2 Receptor Antagonist)
FK506 (Tacrolimus)
Rapamycin (Sirolimus)



Islet transplant activity (1999-2002)

Edmonton (38)

Miami (15)

Minneapolis

NIH (6)

Northweste

Philadelphia

Harvard (4)

Houston (4)

St Louis (4)

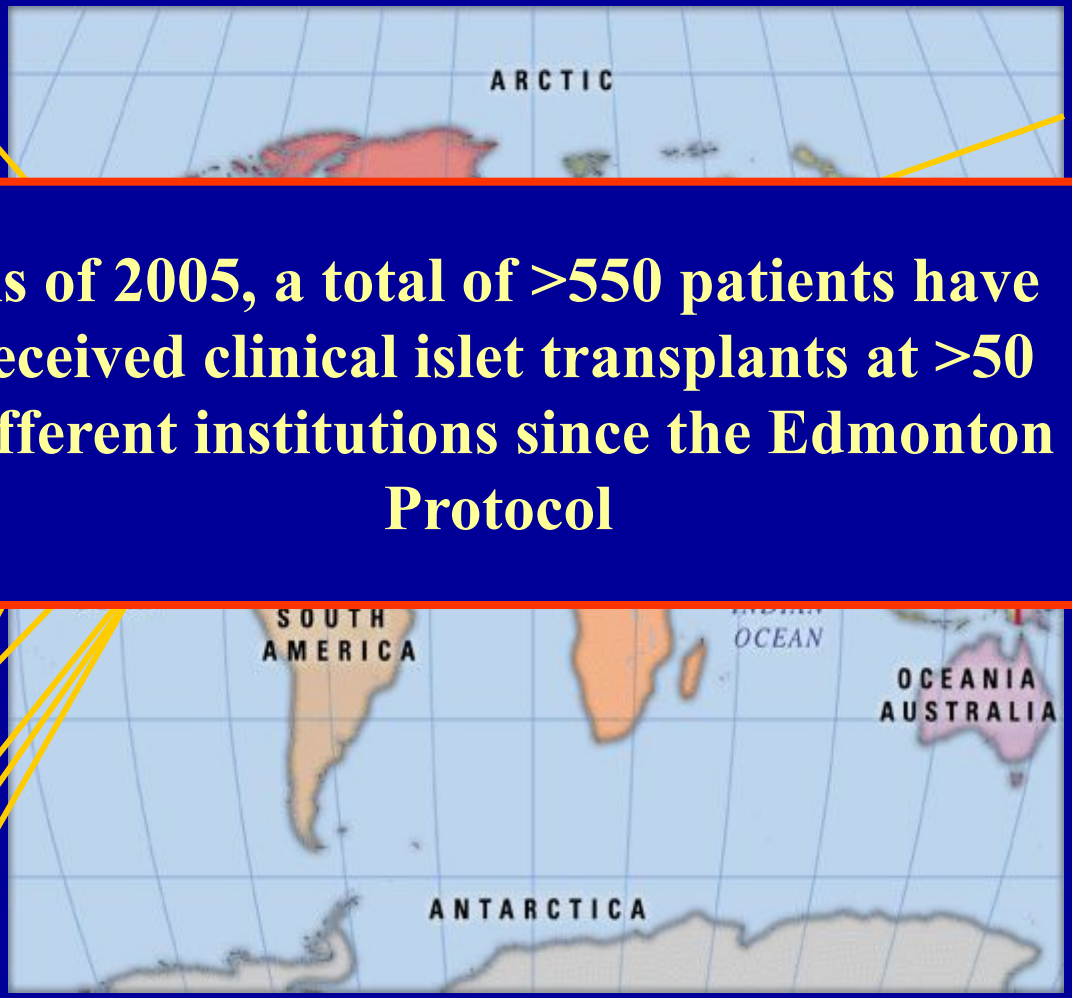
Cincinnati (5)

U. Maryland (1)

Seattle (2)

U Mass (2)

Memphis (2)



Uppsala (5)

eva (14)

n (12)

ch (3)

sen (8)

gs (UK) (1)

As of 2005, a total of >550 patients have received clinical islet transplants at >50 different institutions since the Edmonton Protocol

Long Term Goal

To Treat All Diabetics with Islet Transplantation

Health Canada predicts 6000 new Type 1 diabetics by 2005

Canada: only 400 pancreases available

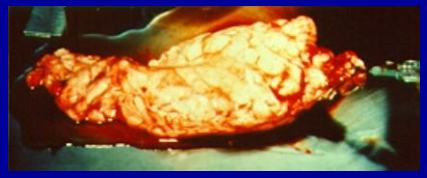
Need Unlimited Source of Islets and No Immunosuppressive Drug Therapy

Criteria of Pig as Donor

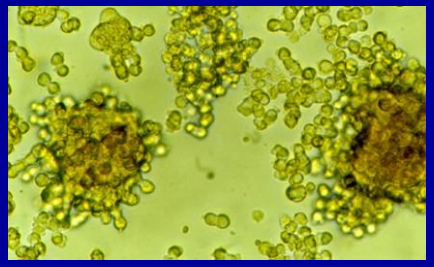
- Breed more rapidly and have large litters
- Inexpensive and readily available
- Exhibit morphological and physiological characteristics comparable to humans
- Pig insulin is structurally similar to human insulin

Alternate Xenogeneic Sources of Tissues for Human Islet Transplantation

Adult Pig



100 – 150 grams

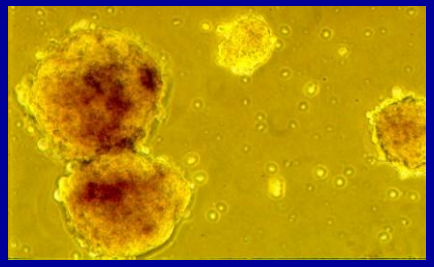


1 day culture

- Islets fragile
- No Growth potential

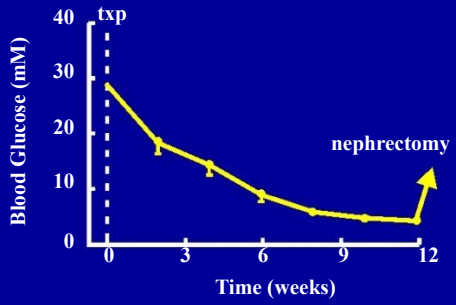
Newborn Pig

2 – 4 grams



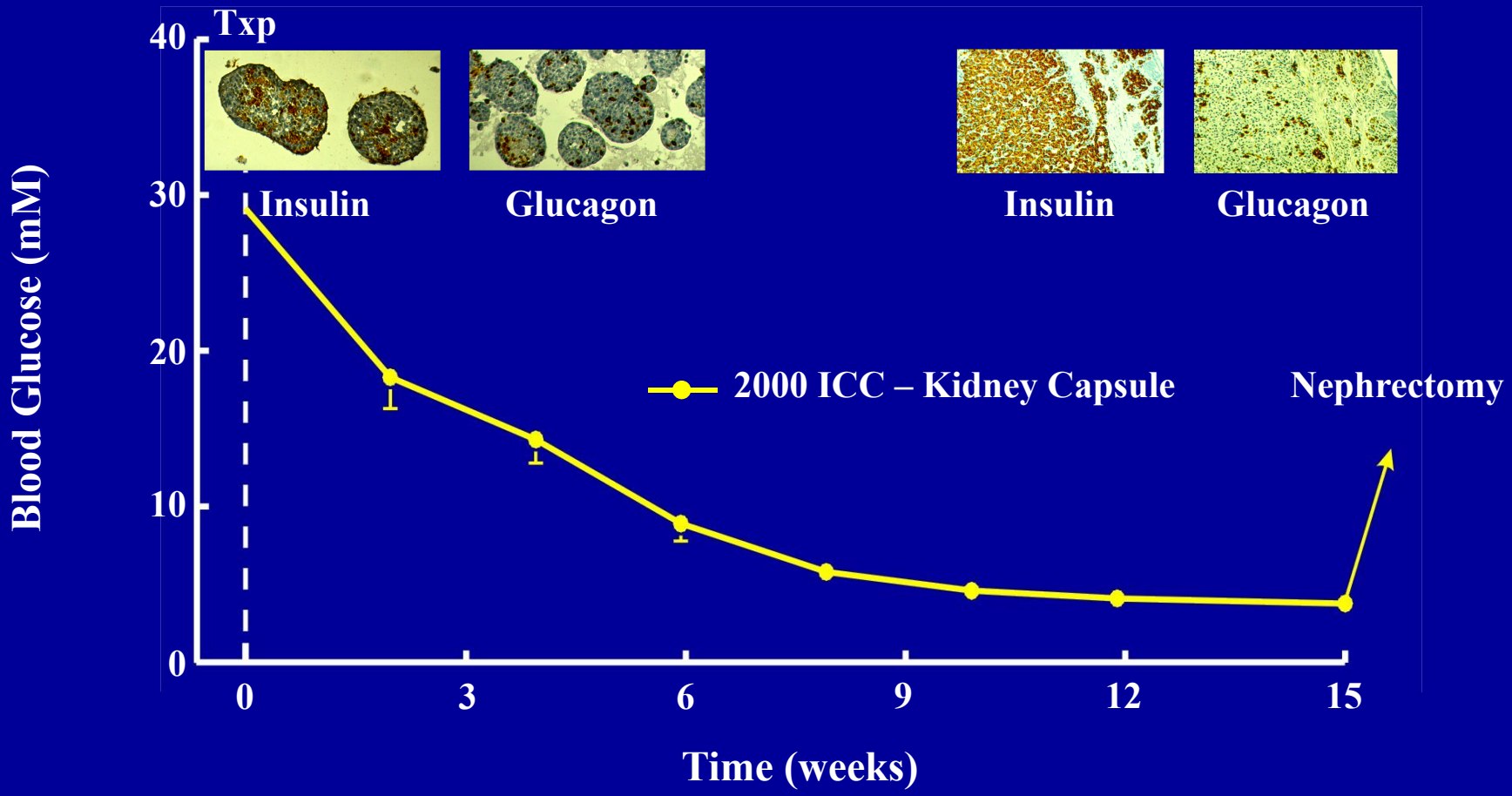
9 day culture

- Islets robust
- Reproducible
- Growth potential



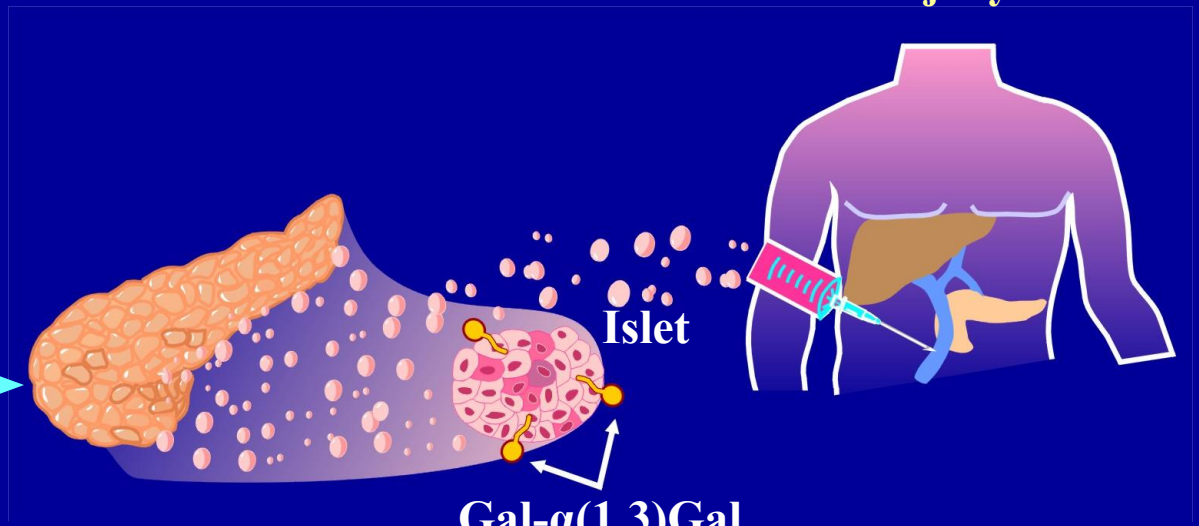
Large Scale Isolation, Growth, and Function of Porcine Neonatal Islet Cells

Gregory S. Korbutt,* John F. Elliott,‡ Ziliang Ao,* Dean K. Smith,‡ Garth L. Warnock,* and Ray V. Rajotte*
**Surgical-Medical Research Institute and ‡Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta T6G 2N8 Canada*

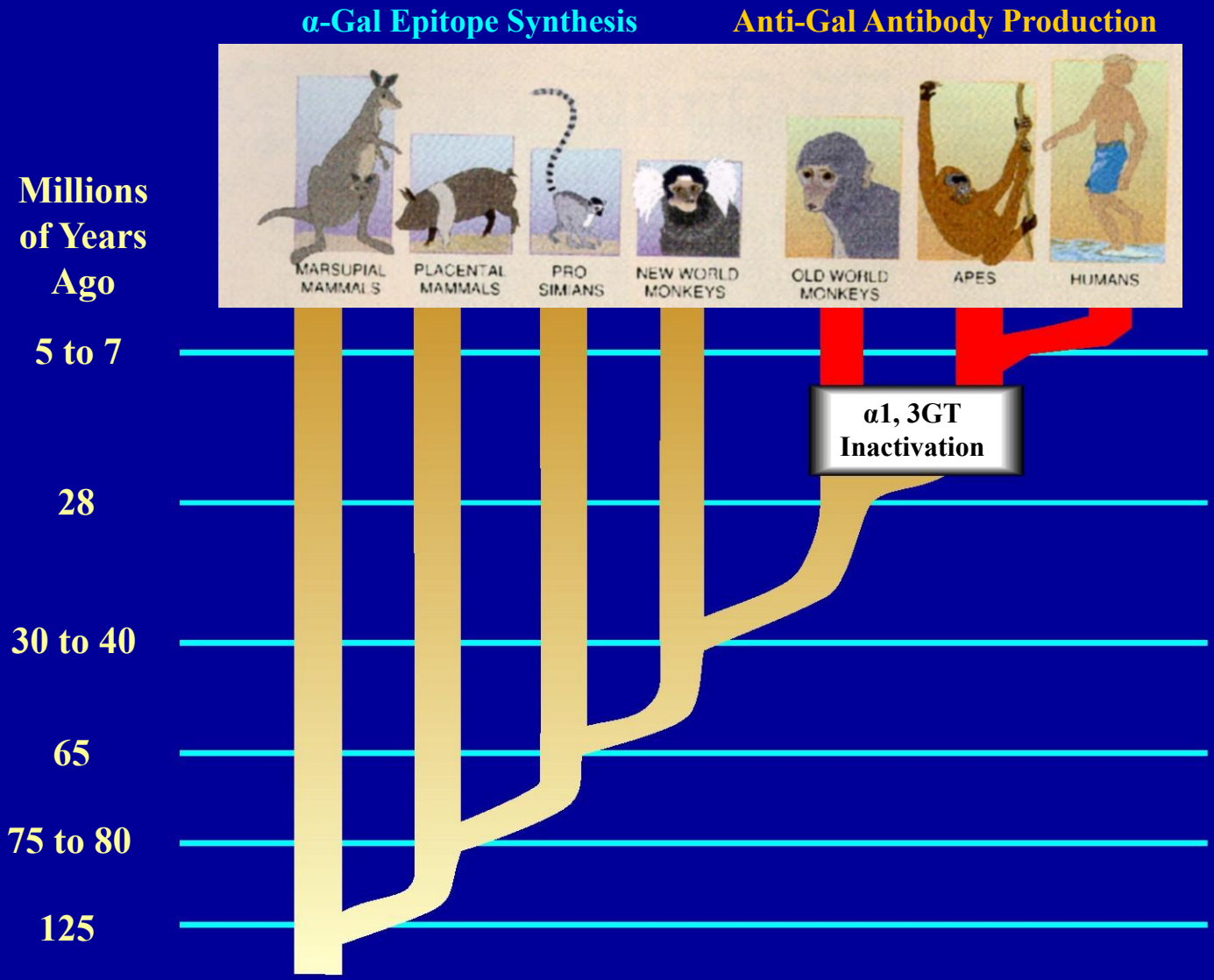


Immunological Responses to Newborn Pig Islets

Newborn Pig

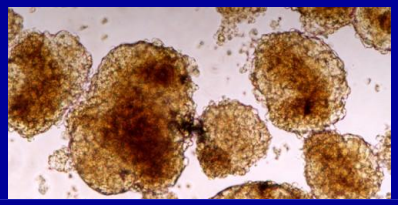


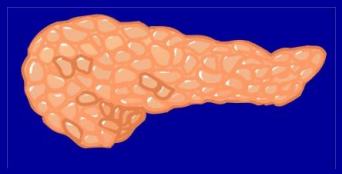
Reciprocal Evolution of Gal α(1,3)Gal Epitopes and the Anti-Gal Antibody in Mammals

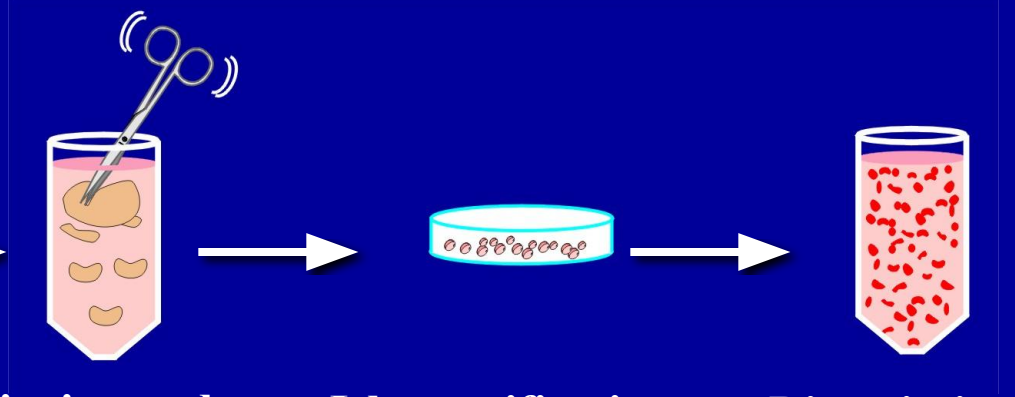


Galili, Uri. Anti-Gal Antibody Prevents Xenotransplantation, Science & Medicine, Vol.5, No. 5 1998, p30

Expression of Galα(1,3)Gal on Newborn Pig Pancreatic Tissues







Surgical removal of pancreas

Mincing and collagenase digestion

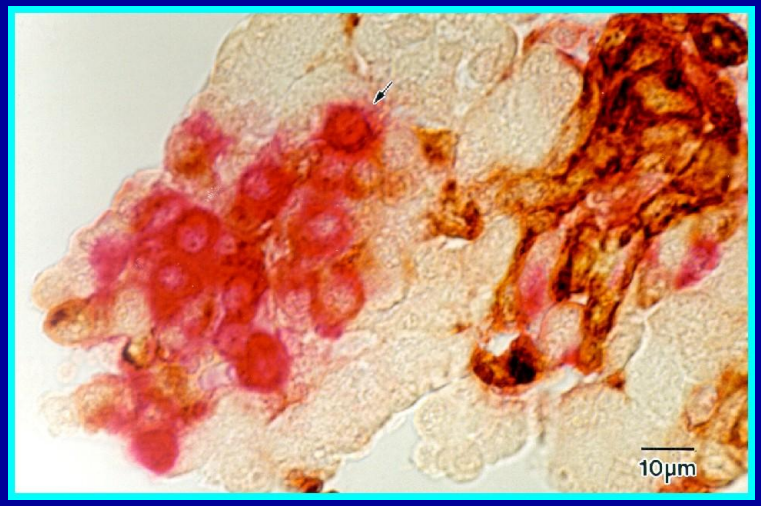
Islet purification (9 day culture)

Dissociation into single cells

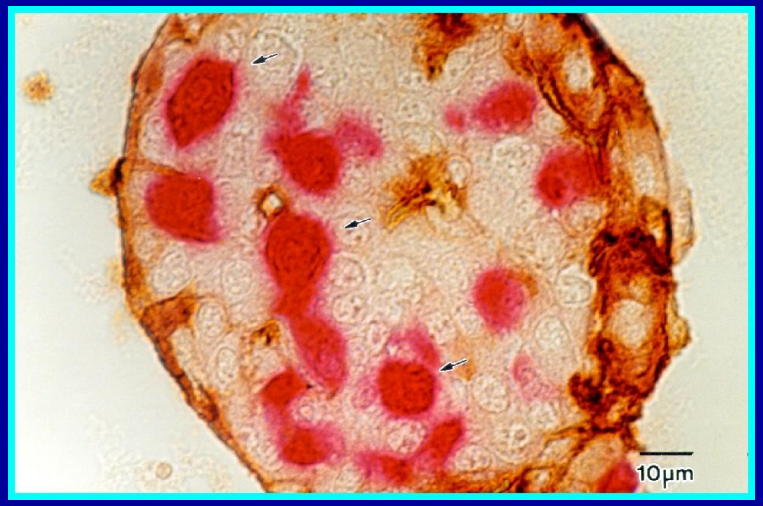
Immunocytochemistry

- Galα(1,3)Gal / Insulin

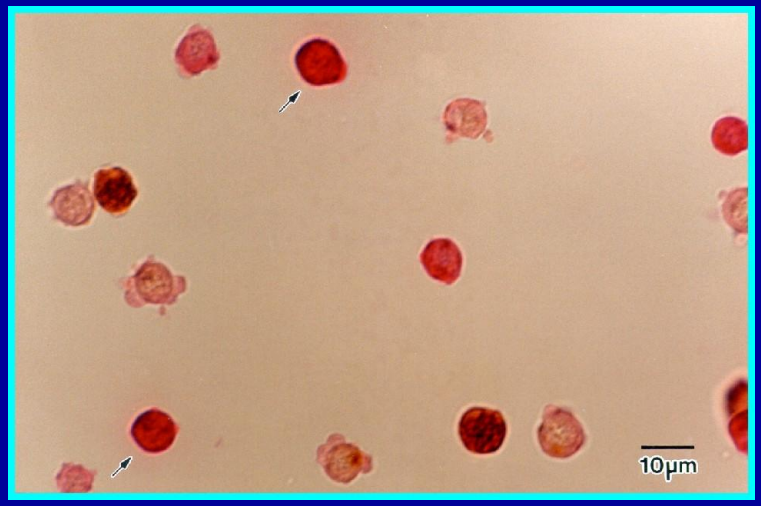
Newborn Pig Pancreas



Collagenase-Digested
Newborn Pig Pancreas

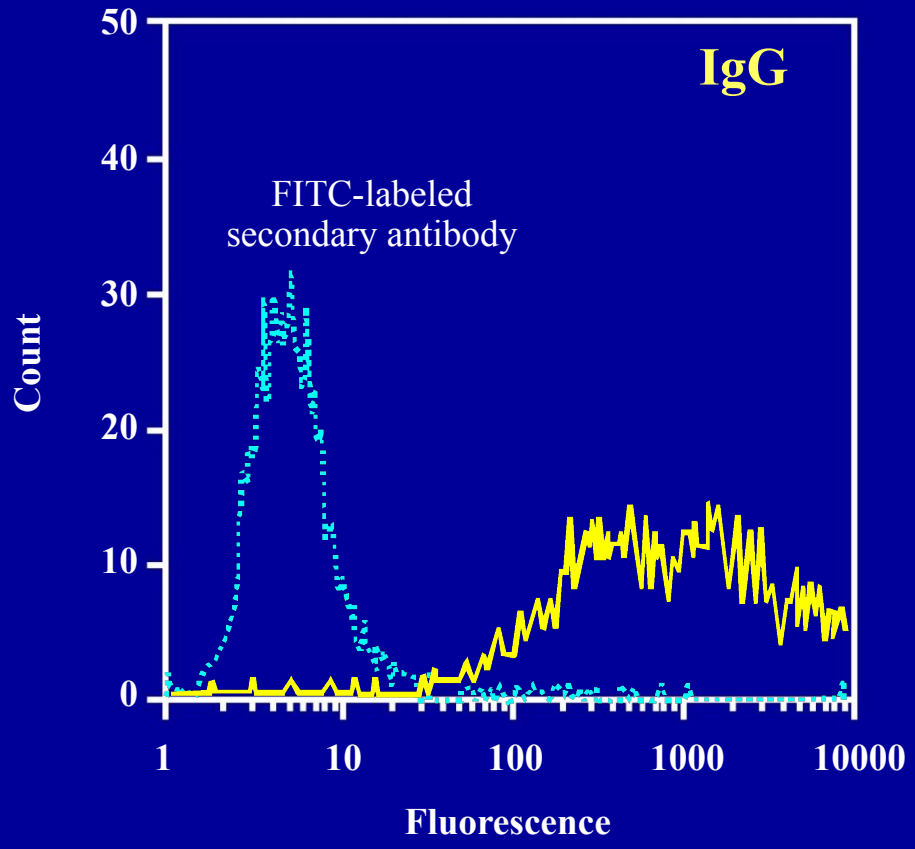


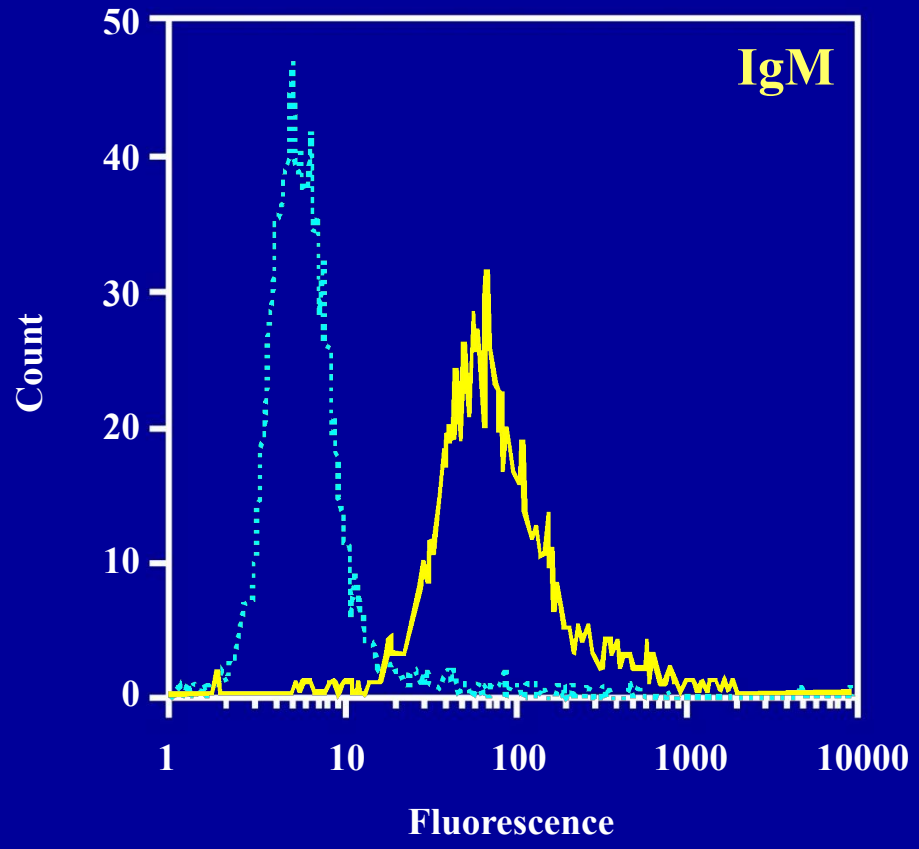
Nine Day-Culture Newborn
Pig Islet



Newborn Pig Islet Cells

Binding of Human Xenoreactive Antibodies to Newborn Pig Islet Cells





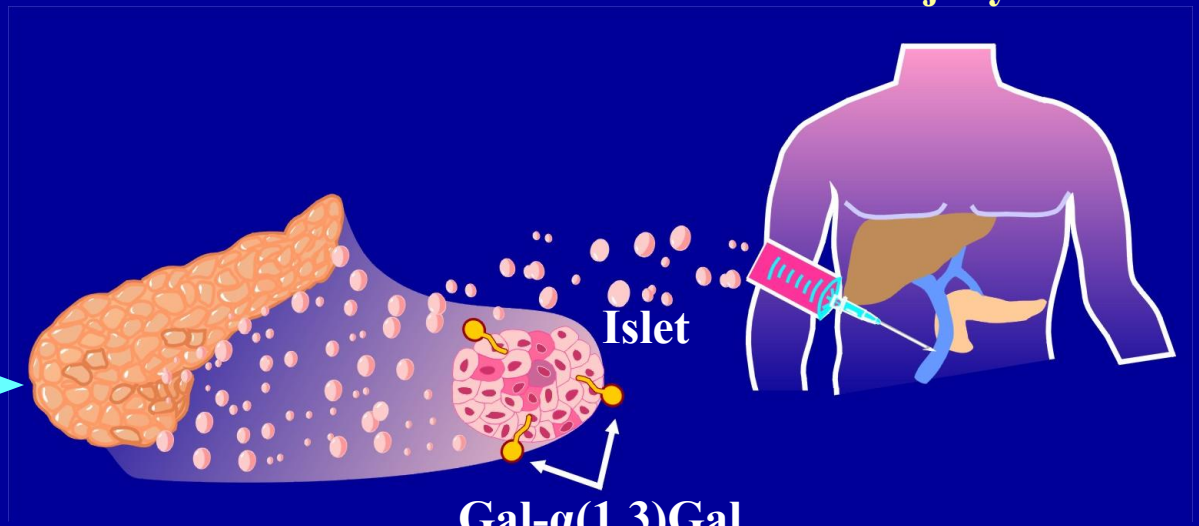
Susceptibility of Newborn Pig Islets to Human Antibody / Complement Mediated Lysis

Condition	Percentage of Dead Cells (n)		
	Unsorted	Gal positive	Gal negative
Heat-inactivated pooled AB serum + complement	97.4±1.7 (9)	97.9±1.3 (11)	97.9±1.1 (15)
Complement	92.5±3.1 (9)	91.4±3.0 (10)	90.9±2.9 (14)
Fresh AB Serum	40.7±4.9* (6)	46.9±7.7* (4)	45.7±3.2* (6)

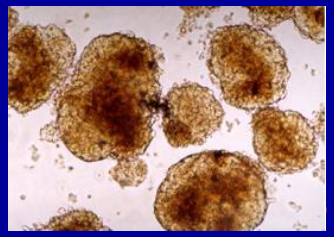
Data are means ± SE of (n) independent experiments. Cells were exposed to 50% human serum for 1 h and 20% rabbit complement for another 30 min. Fresh serum was from one individual with blood type AB. * $p<0.05$ vs. complement or heat-inactivated-pooled AB serum + complement, ANOVA.

Immunological Responses to Newborn Pig Islets

Newborn Pig



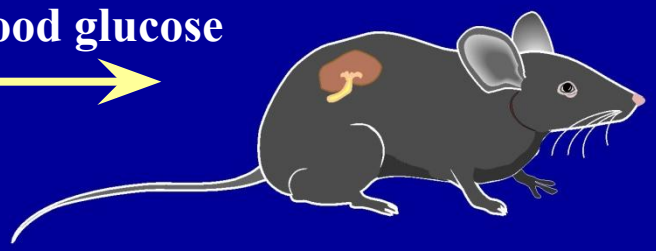
Experimental Design



Newborn pig islets transplant into

STZ-induced diabetic B6 or B6 *Rag-/-* mouse

Monitor blood glucose

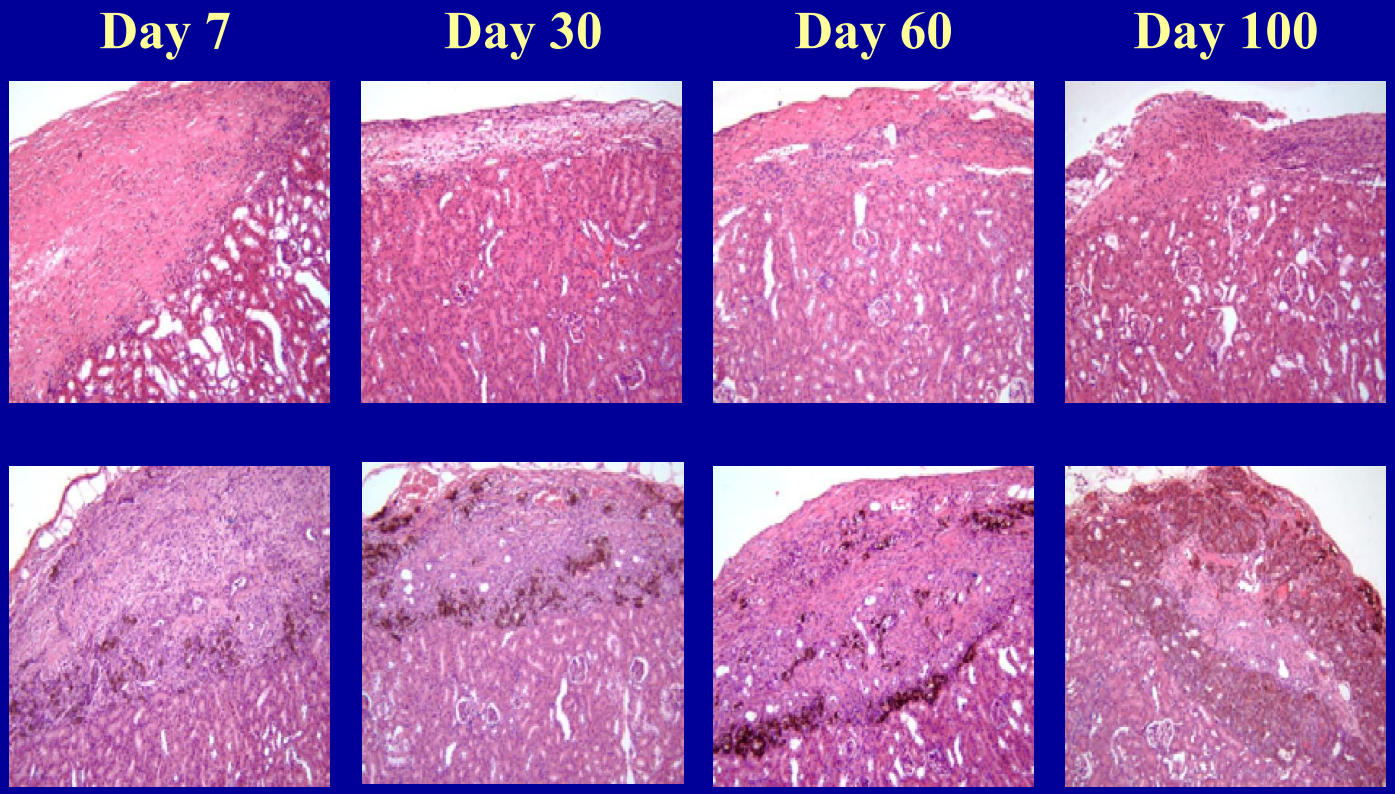


Diabetic B6 or Normoglycemic B6 *Rag-/-* mouse

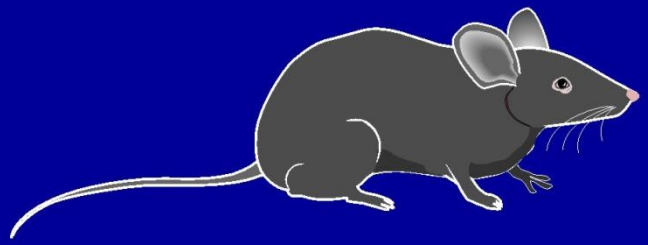
Immunohistochemistry

on days 7, 30, 60, 100 post-transplantation

Newborn Pig Islet Grafts in Mice

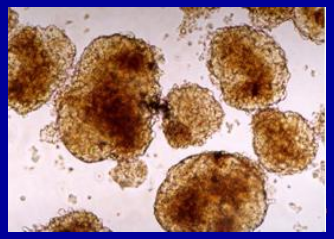


Experimental Design

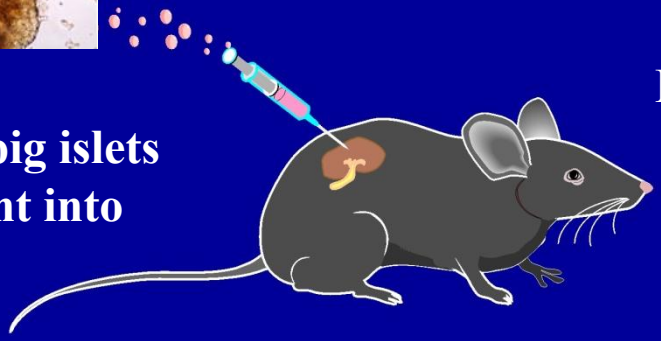


Naïve B6 mouse

Isolate lymph node cells;
$CD4^+$ or $CD8^+$ T cells
($10x10^6$)



Newborn pig islets
transplant into

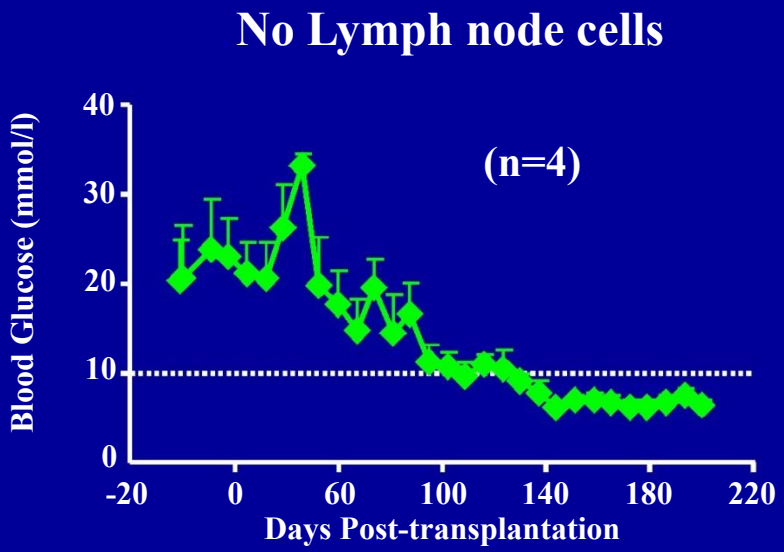


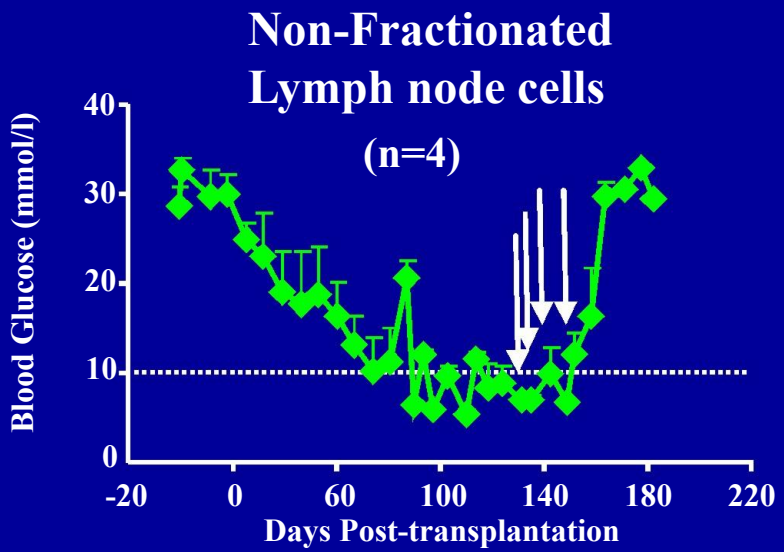
STZ-induced diabetic
B6 *Rag-/-* mouse

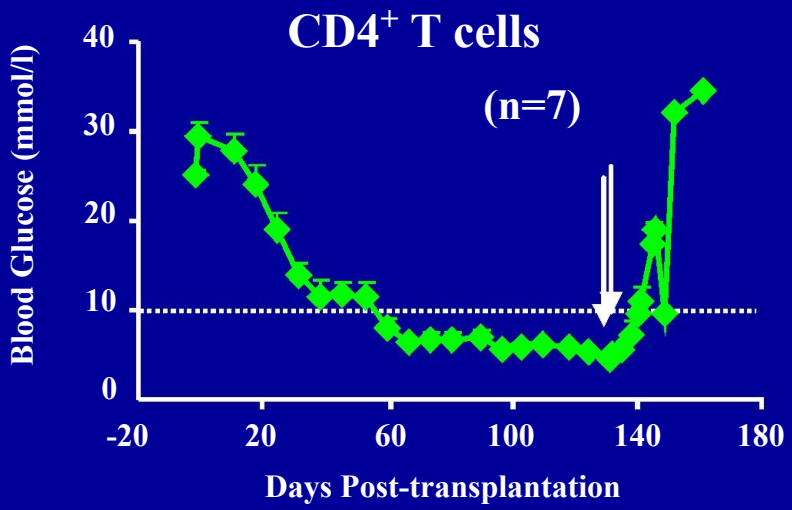
Monitor blood glucose
(>100 days)

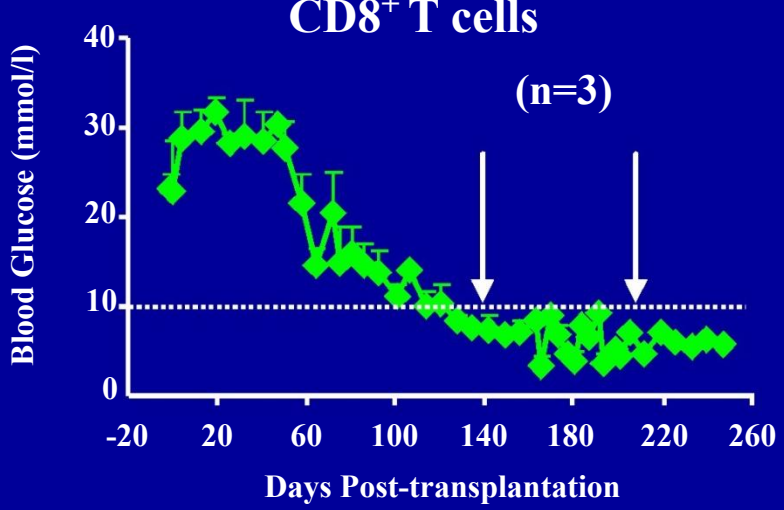
Normoglycemic
B6 *Rag-/-* mouse

Rejection of Newborn Pig Islets by T cells









Monoclonal Antibodies

Anti-CD154 antibody

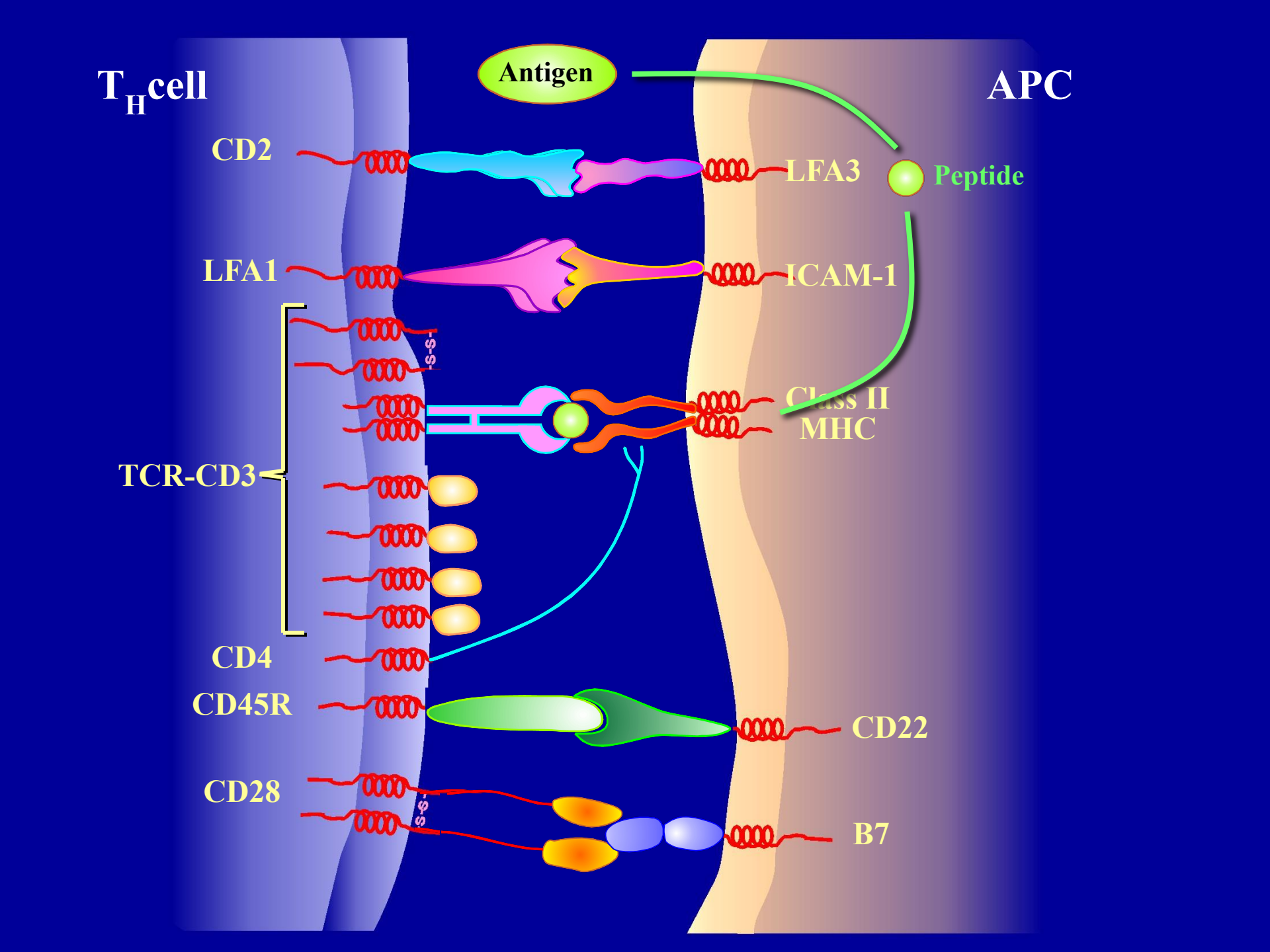
Parker DC, et al. Proc Natl Acad Sci USA 1995; 92: 9560-4.
Kenyon NS, et al. Proc Natl Acad Sci USA 1999; 96: 8132-7.
Markees TG, et al. Transplant Proc 1996: 814-5.
Wijkstrom M, et al. Xenotransplantation 2001; 8 (Suppl 1) #526.
Appel MC, et al. Transplantation 2004; 77: 1341-9.

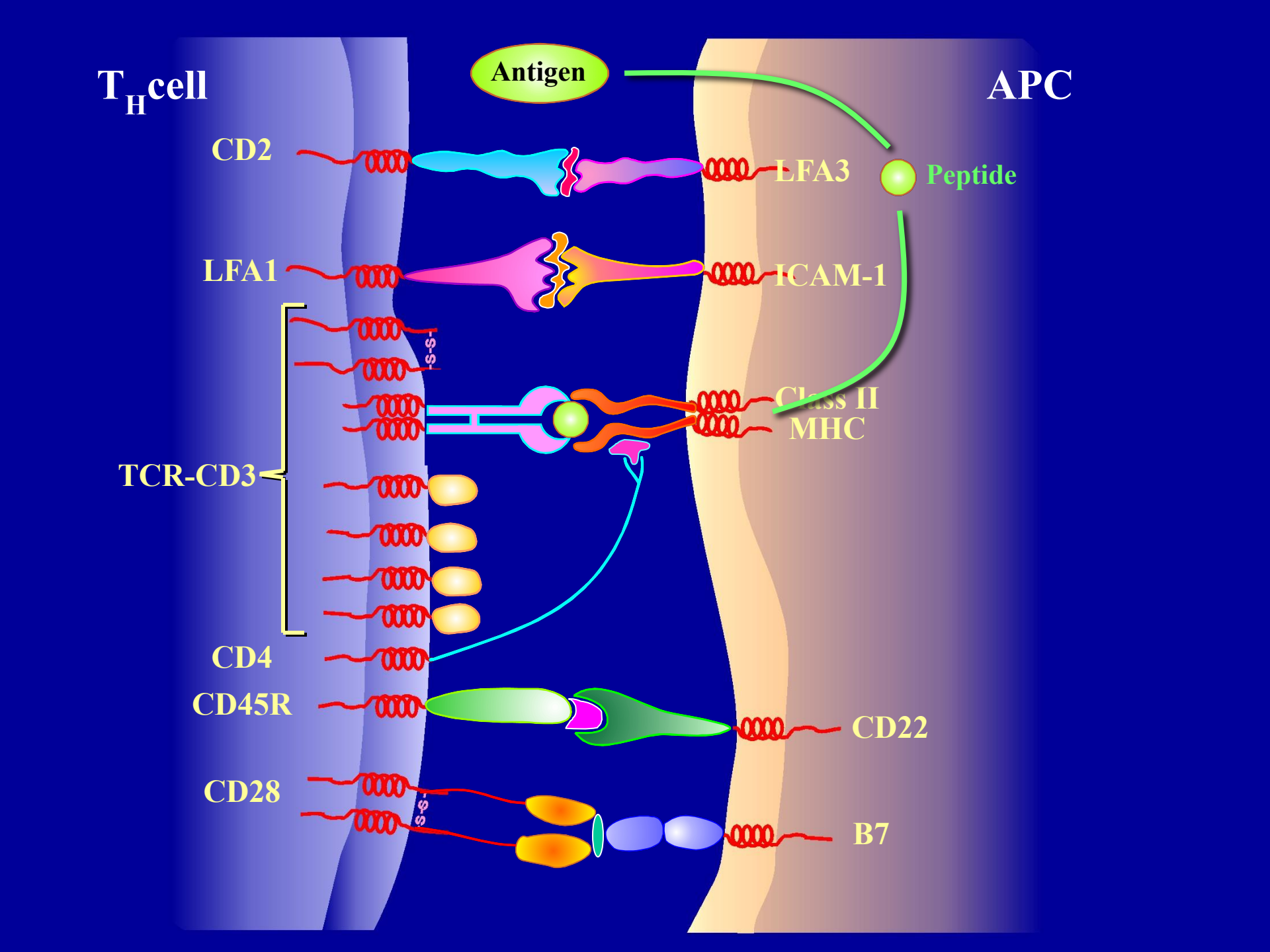
Anti-LFA-1 antibody

Nicolls MR et al. J Immunol 2000; 164: 3627-34.
Beilke J and Gill RG. Xenotransplantation 2001; 8 (Suppl 1) #023.

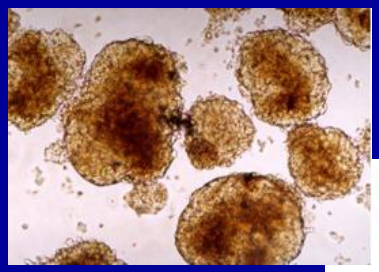
Anti-CD45RB antibody

Kalayanamit T, et al. Xenotransplantation 2001; 8 (Suppl 1) #024.
Deng S, et al. Xenotransplantation 2001; 8 (Suppl 1) #025.

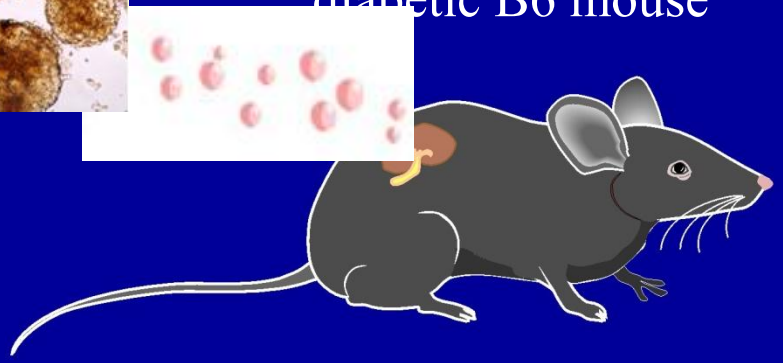

$T_H$cell
Antigen
APC
CD2
LFA3
Peptide
LFA1
ICAM-1
Class II
MHC
TCR-CD3
CD4
CD45R
CD22
CD28
B7


$T_H$cell
Antigen
APC
CD2
LFA3
Peptide
LFA1
ICAM-1
Class II
MHC
TCR-CD3
CD4
CD45R
CD22
CD28
B7

Experimental Design



Newborn pig islets,
transplant under
the kidney capsule of
diabetic B6 mouse



Treatment

- Rapamycin
- Anti-LFA-1
- Anti-CD154
- Anti-CD45RB
- Combination of the above therapies

- Monitor blood glucose
- Harvest islet graft for immunohistochemical analysis
- Blood serum for mouse anti-pig antibodies

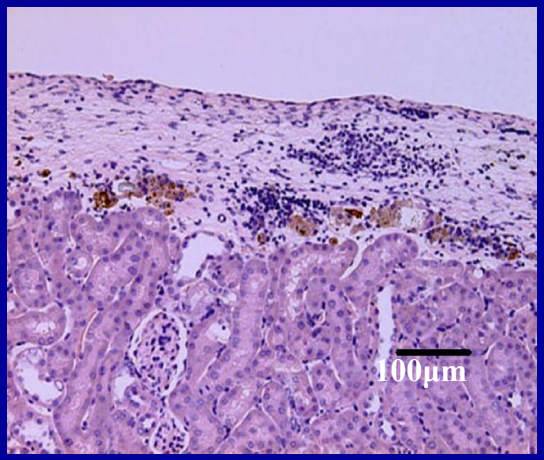
Survival of NPI Xenografts in B6 Mice Treated with Various Anti-rejection Therapies

Treatment	n	Graft Survival (days post-tx)*	# of Mice Achieving Normoglycemia
Rapamycin	7	0(x7)	0/7
Anti-LFA-1*	15	0(x8), 86, >100(x6)	7/15
Anti-CD45RB	8	0(x4), 67, >100(x2)	3/8
Anti-CD154	7	0(x4), 83, >100(x2)	3/7
Rapamycin + Anti-LFA-1	10	0(x7), 99, >100(x2)	3/10
Anti-LFA-1 + Anti-CD154*	14	0, 0, >100(x12)	12/14
Anti-LFA-1 + Anti-CD45RB	12	0(x4), 82, >100(x7)	8/12

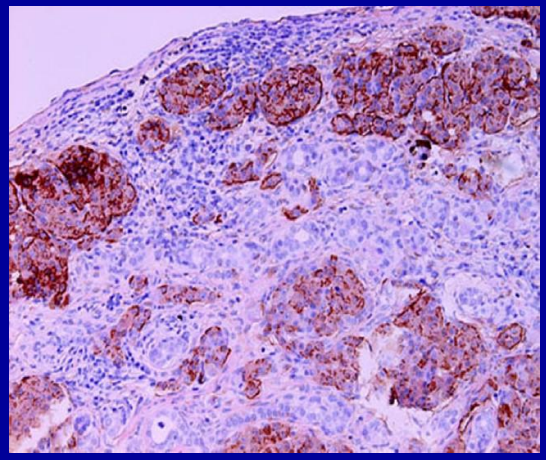
* Graft survival of 0: mice did not achieve normoglycemia post-tx.
* $p<0.04$ vs rapamycin
* p=0..01 vs rapamycin + anti-LFA-1

Effects of Rapamycin and Monoclonal Antibody Therapy on the Survival of Newborn Pig Islet Xenografts

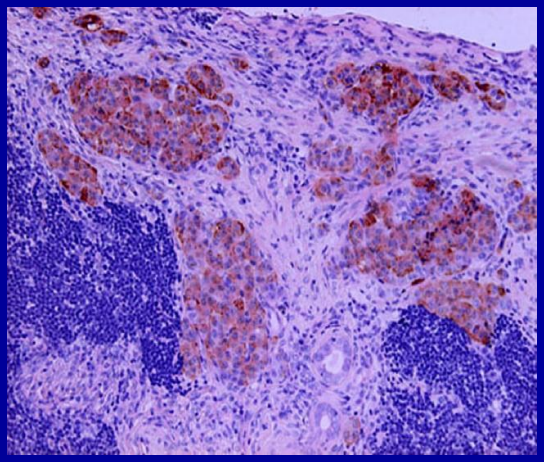
Rapamycin



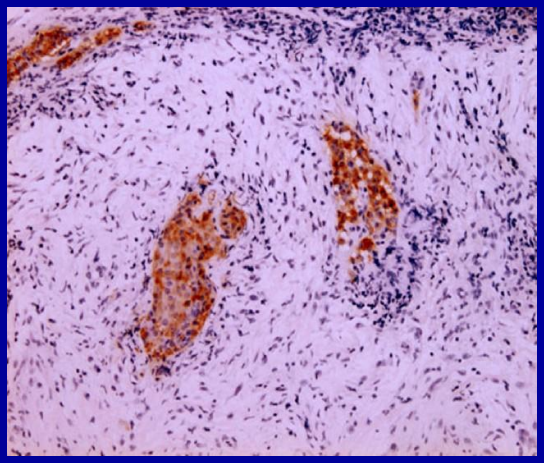
Anti-LFA-1



Anti-CD154



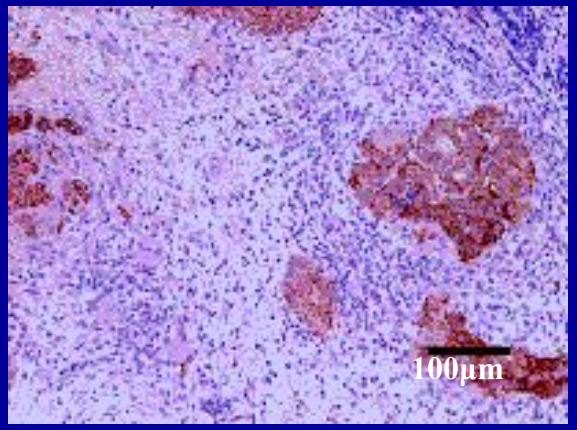
Anti-CD45RB



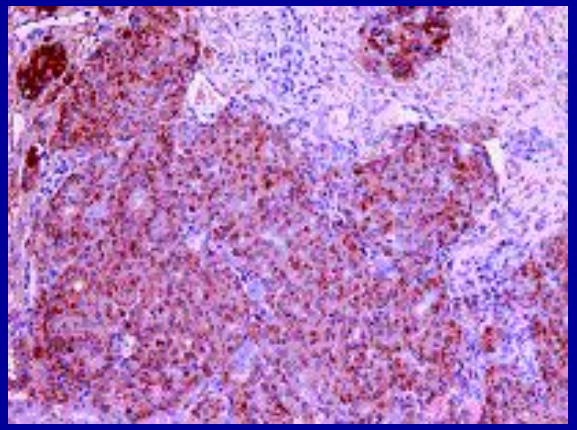
Insulin positive cells (brown stain)

Combination of Anti-LFA-1 with Anti-CD154 or Anti-CD45RB Monoclonal Antibodies Induces Long-Term Islet Xenograft Survival in B6 Mice

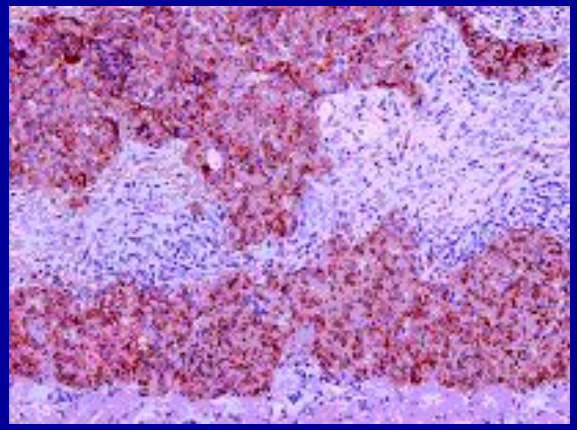
Rapamycin + Anti-LFA-1



Anti-CD154 + Anti-LFA-1

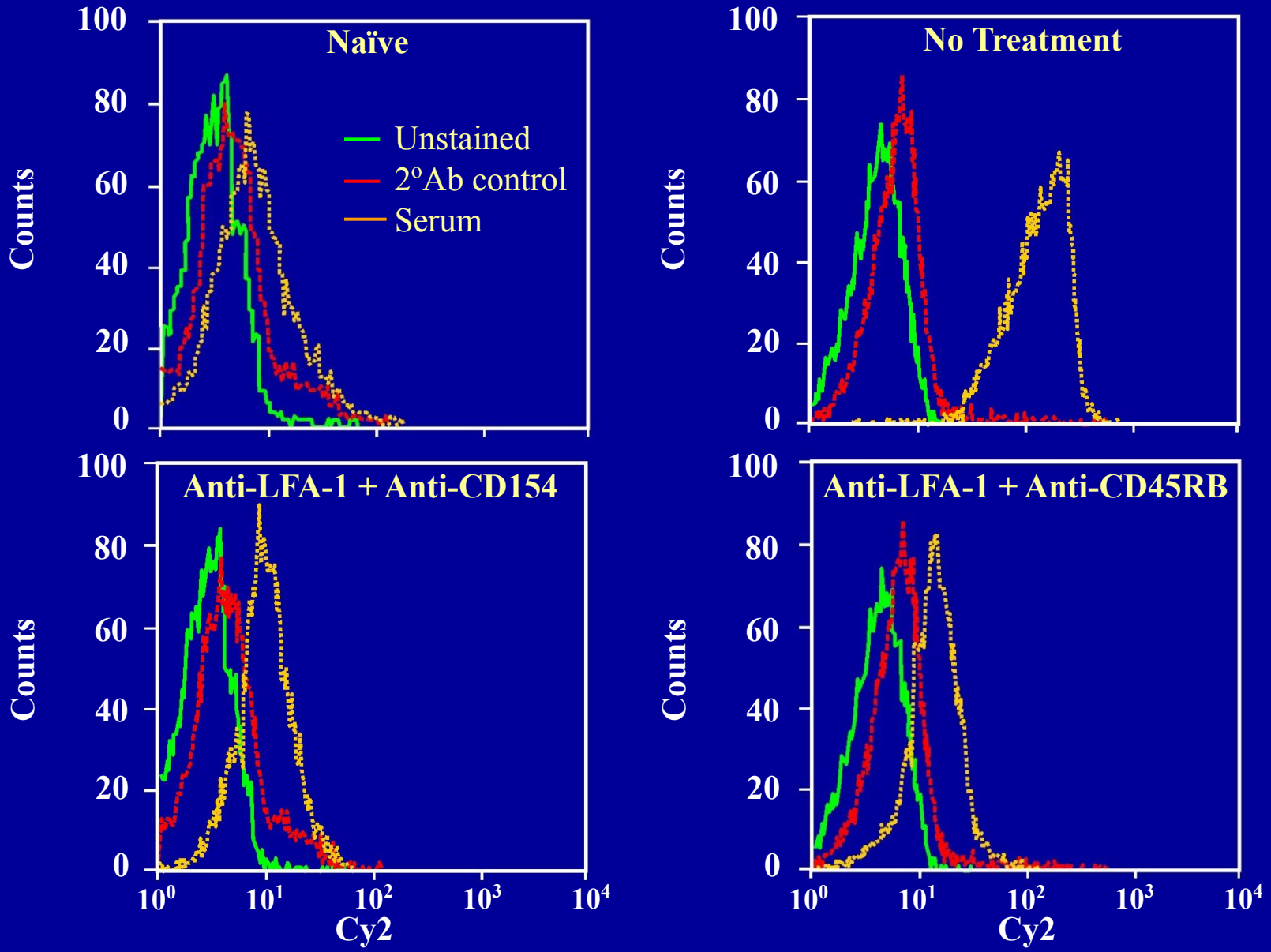


Anti-CD45RB + Anti-LFA-1

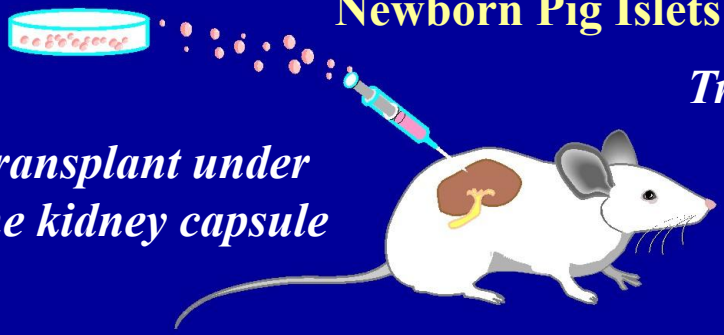


Insulin positive cells (brown stain)

Combination of Anti-LFA-1 with Anti-CD154 or Anti-CD45RB Inhibits the Production of Anti-Pig Antibodies in B6 Mice



Experimental Design



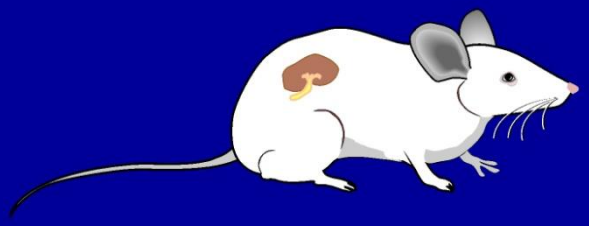
Newborn Pig Islets

Transplant under the kidney capsule

Diabetic NOD mice

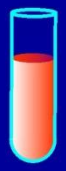
Treat with anti-rejection therapies

Monitor Blood glucose



100 days Post-Tx

Immunohistochemistry



FACS: Anti-pig antibodies

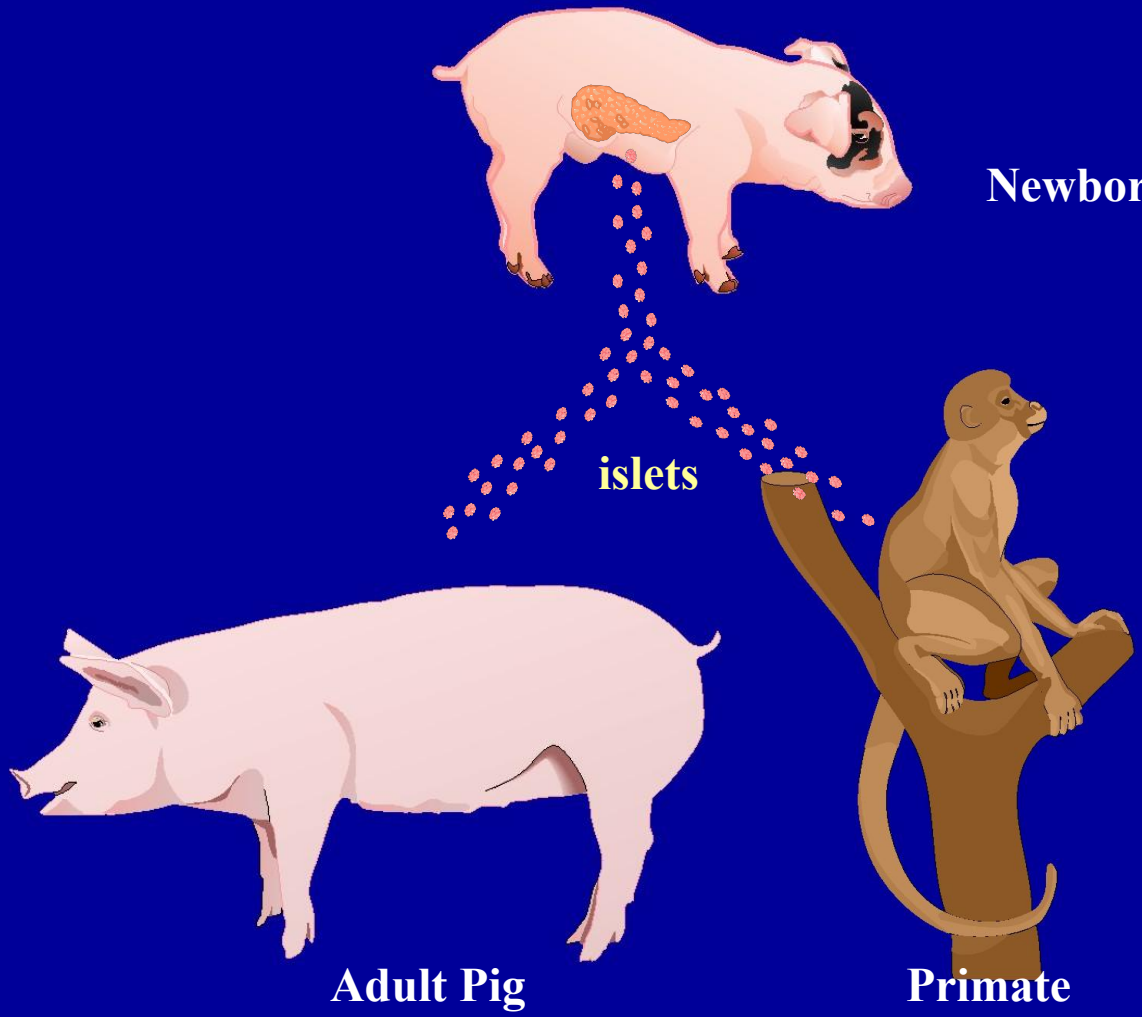
Efficacy of Combinations of Monoclonal Antibodies in Promoting Survival of NPI Xenografts in NOD Mice

Monoclonal Antibody	n	Graft Survival (days post-tx)
Anti-LFA-1+anti-CD154*	2	0x2
Anti-LFA-1+anti-CD154*	7	>100, >100, 0x4, 65
Anti-LFA-1+anti-CD154*+anti-CD4	5	72, 42, >100, >100, 56
Anti-CD4	5	0x5

* Short regimen as in B6 mice

* Long regimen (injection of MR-1 and anti-LFA-1 mAbs was for 10 weeks post-transplantation)

Islet Xenotransplantation in Preclinical Models



Reversal of Diabetes in Pancreatectomized Pigs after Transplantation of Neonatal Porcine Islets

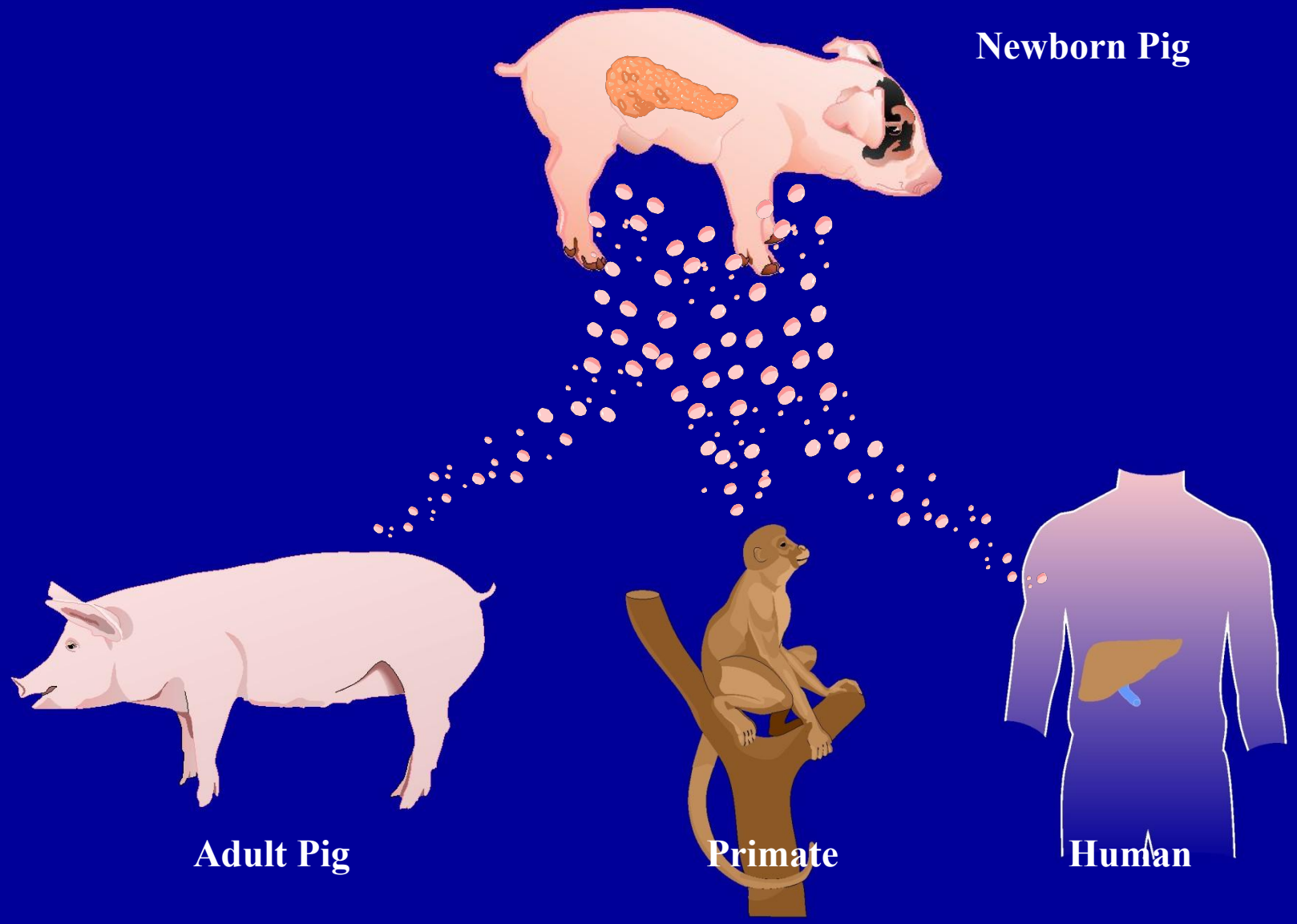
Tatsuya Kin,[1,2] Gregory S. Korbutt,[1,2] Tsunehiro Kobayashi,[1,2] Jannette M. Dufour,[1,2] and Ray V. Rajotte[1,2,3]

DIABETES, VOL. 54, APRIL 2005

Neonatal porcine islets (NPIs) are able to grow and to reverse hyperglycemia after transplantation in immunoincompetent mice. The aim of this study was to demonstrate the feasibility of allogeneic NPIs grafts to achieve normoglycemia in a pancreatectomized diabetic pig. NPIs were isolated from pancreases of 1- to 3-day-old pigs, cultured, and then transplanted via the portal vein into the liver of totally pancreatectomized pigs

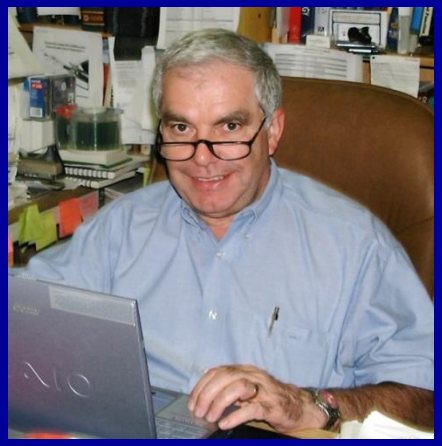
logical features with humans, breeds rapidly, and produces large litters. Porcine insulin has also been used successfully for a long time to treat diabetic patients. Adult porcine islets, however, have been difficult and expensive to obtain on a consistent basis with the additional problem that, once isolated, adult porcine islets are fragile and difficult to maintai

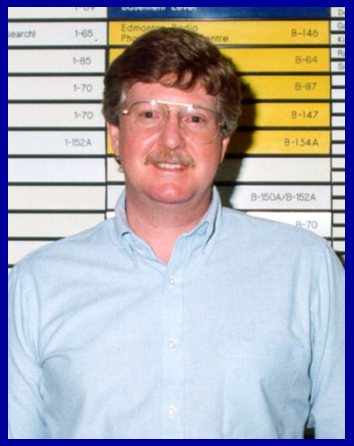
Future Application of Islet Xenotransplantation



Acknowledgments

1. Canadian Diabetes Association
2. Stollery for Children Hospital Foundation
3. Edmonton Civic Employees Charitable Assistance Fund
4. Dr. Ray V. Rajotte, Dr. Gregory S. Korbutt, Dr. Ron G. Gill and their staff





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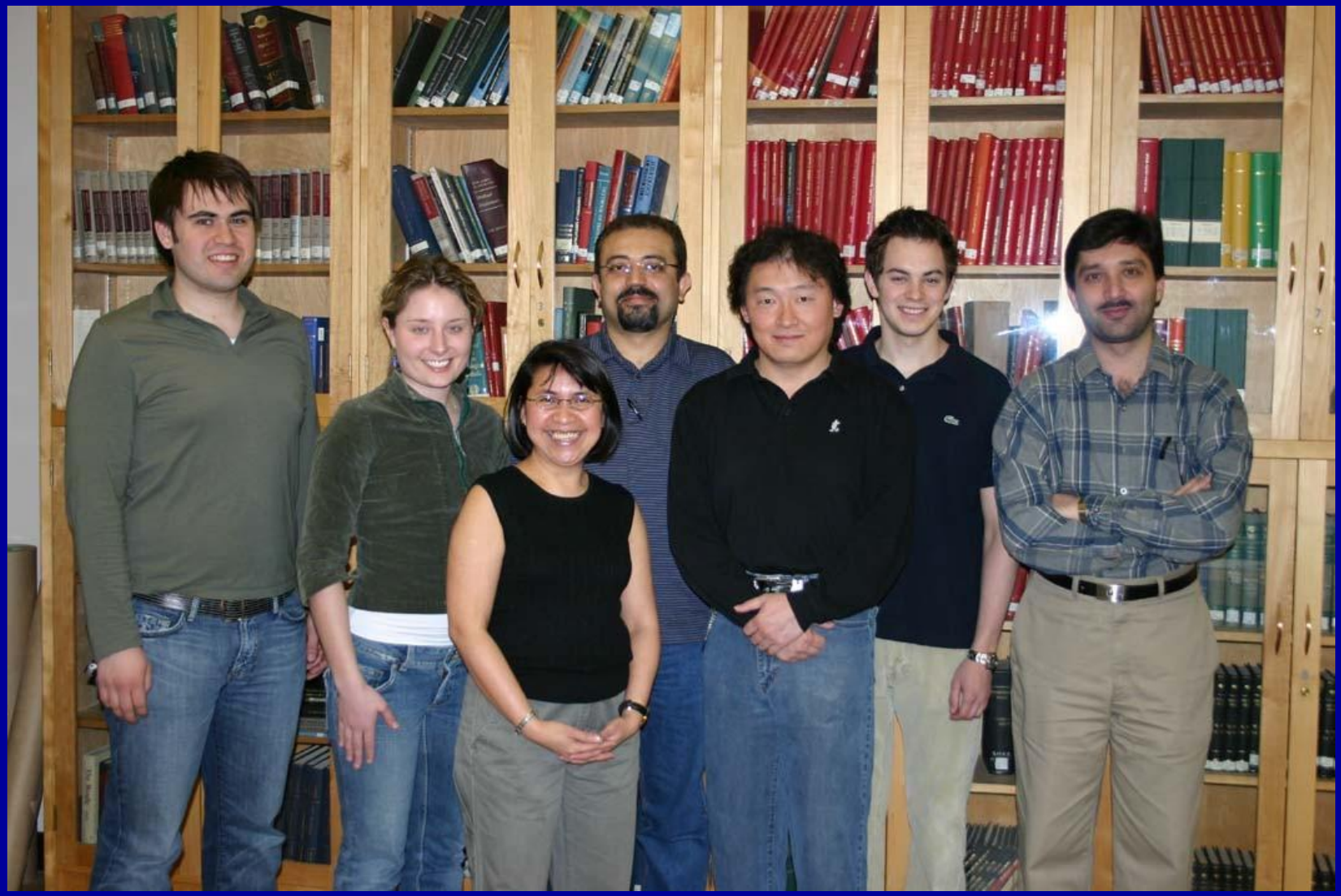

5. My students and fellow

Edmonton River Valley September 2005
Photo:
D. Colwell