Generation of long-term human pancreatic islet cell activity *in vivo* by induction of immune tolerance to human stem cells

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Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins

FIGURE 1.—RAY OWEN around 1960.

- **Intermingling of siblings cells in placental circulation results in long-term chimerism**
- **Immune tolerance is acquired during development**
- **Existence of stem cells and their engraftment**

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No. 4379 October 3, 1953 NATURE 603

'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

By Dr. R. E. BILLINGHAM*, L. BRENT and Prof. P. B. MEDAWAR, F.R.S.

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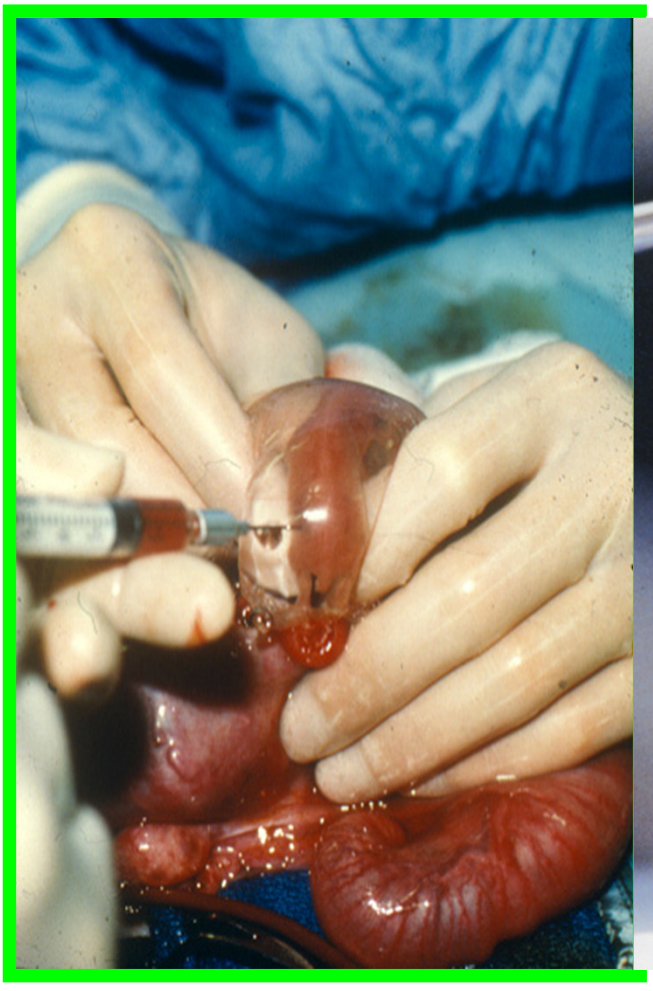
The inoculum from A-line male suspension in ringer's solution of small organized tissue clumps, isolated cells and cell debris prepare by prolonged chopping with scissors of testis, kidney and splenic tissue
0.01 ml injected via intraperitoneal injection into day 15-16 fetuses of a CBA X CBA mating
After birth skin graft acceptance was determined

Summary

- Cellular inoculum during development results in skin graft tolerance in adult life
- The tolerance is donor specific (i.e. full ability to respond to 3rd party donor)

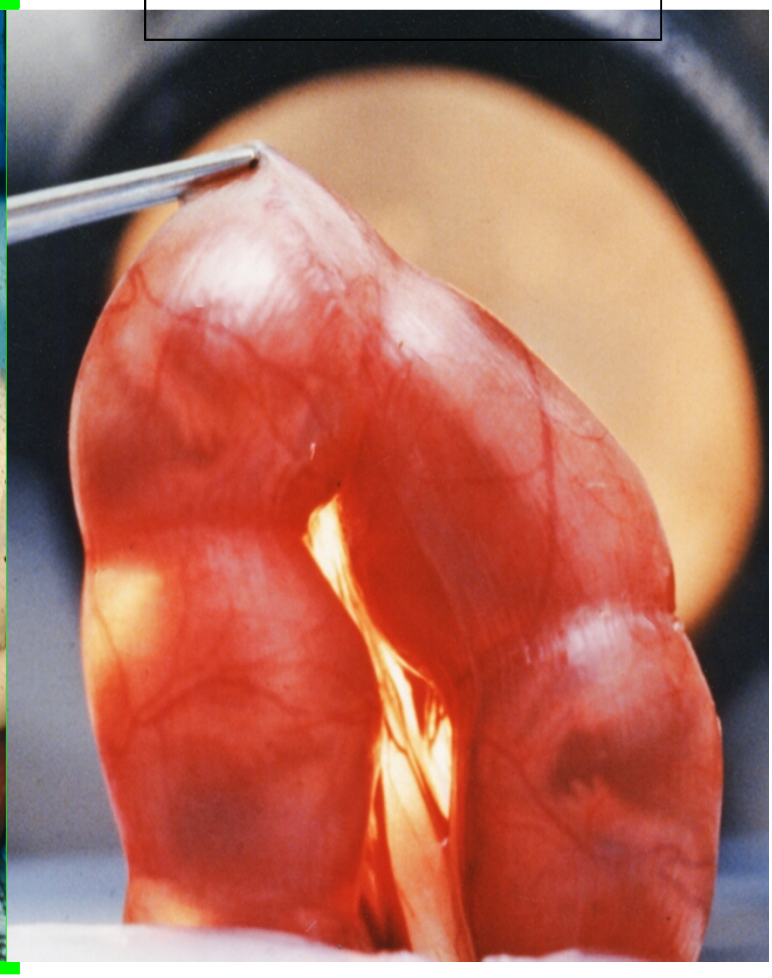
Intrauterine transplantation

IP injection on day 58-65



SHEEP

IP injection on day 14-16



MOUSE

Engraftment and Long-term Expression of Human Fetal Hematopoietic Stem Cells in Sheep Following Transplantation *in Utero*

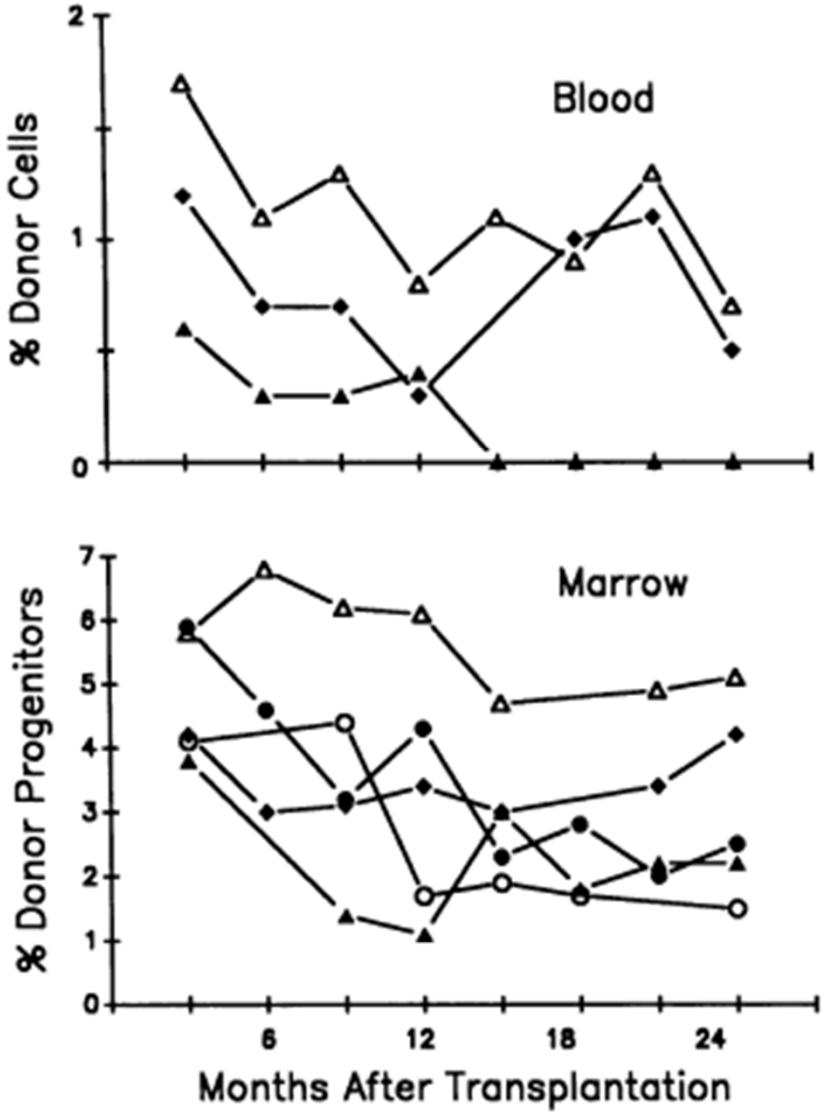
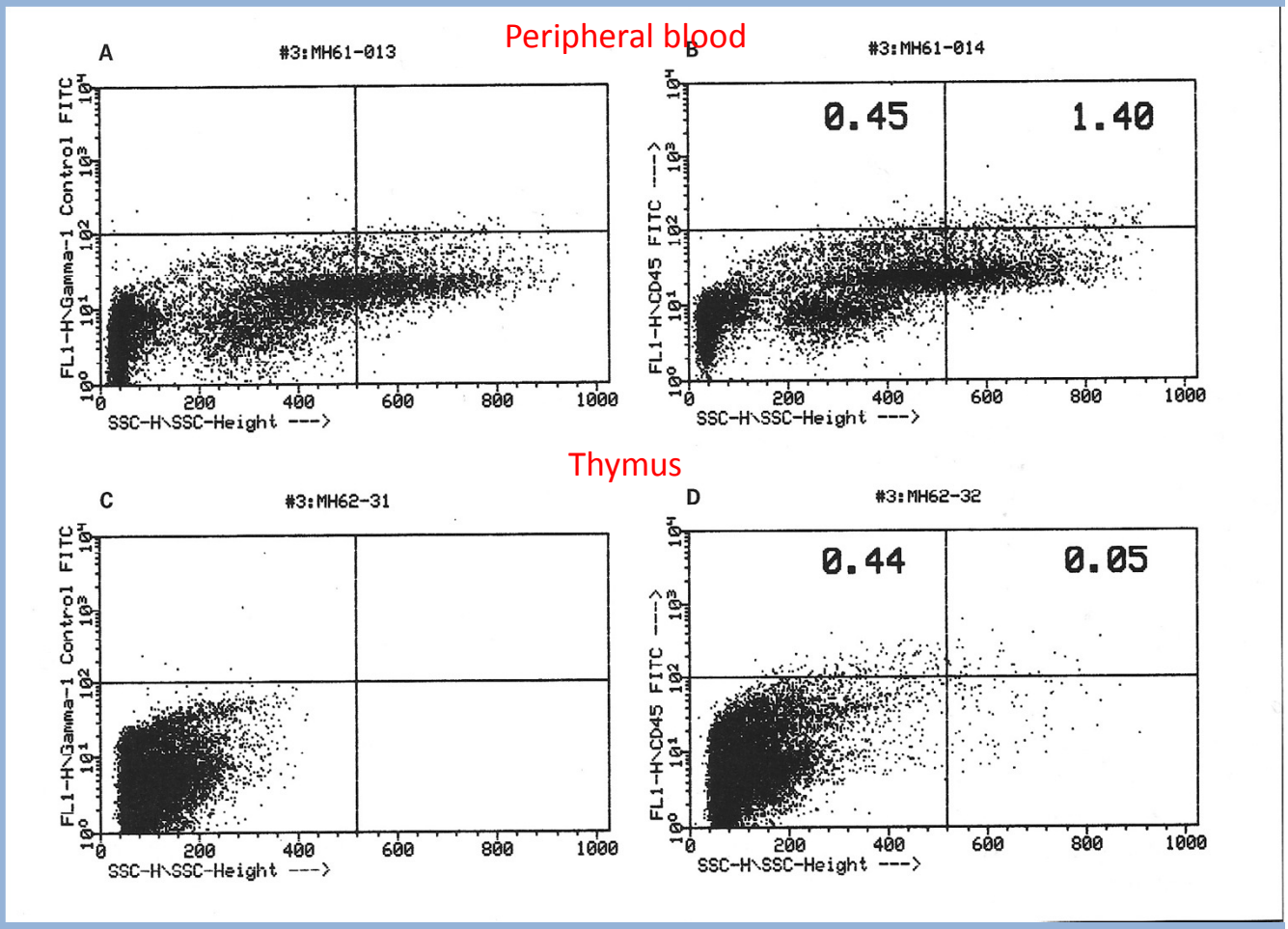


Figure 6. Persistence of donor (human) cells (blood)/progenitors (marrow) in chimeric sheep. Peripheral blood data are provided for the three animals that showed donor cells in circulation at birth. Bone marrow progenitors were assessed as described in text and legends to Fig. 3. ● —— ●, 1219C; ○ —— ○, 1219D; △ —— △, 3419; ▲ —— ▲, 3425; ♦ —— ♦, 3425C.

<u>Fetal age at transplantation day 48-54 term gestation 145 days</u>

J Clin Inv 1992; 89: 1178-88

Prolonged hematopoietic chimerism in normal mice transplanted in utero with human hematopoietic stem cells

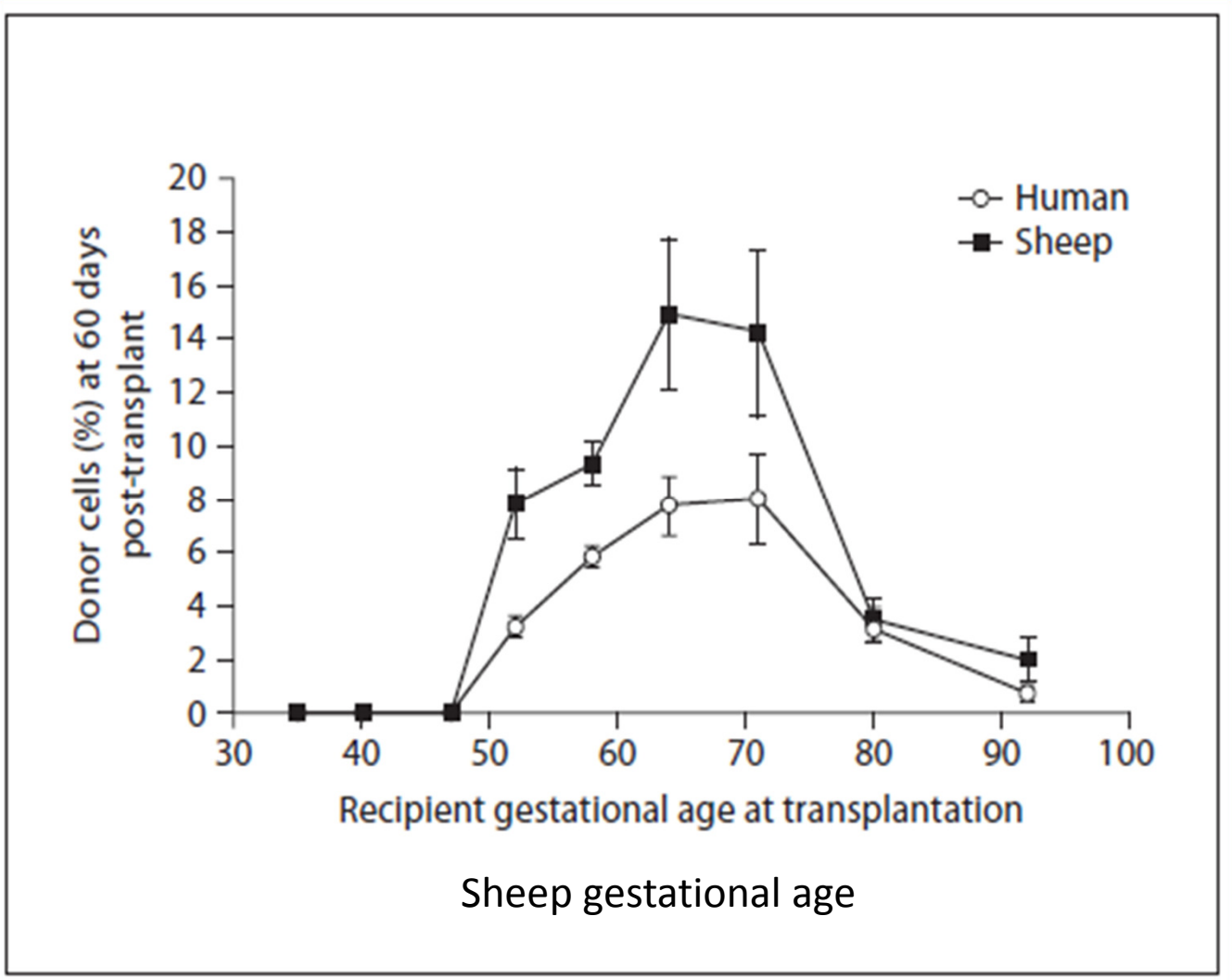


Human CD45

Side scatter

Pathobiology 1998; 66: 230-39

Engraftment receptivity is gestational age dependent



ENGRAFTMENT WINDOW: day 52-72

Fetal Diagn Ther 2009;25:102-110

Murine maternal and fetal viability following in utero transplantation of human HSC

Donor cell population[1]	Gestational age[3], days	Pregnant mice xeno-transplanted	Perioperative mortality	Fetuses injected[2]	Live-born mice[3]
Adult HSC	12–15	42	4 (9.5)	415	98 (24)
Adult HSC	12	25	2 (8)	236	39 (16)
Adult HSC	13–15	17	2 (12)	179	59 (33)
Fetal HSC	12	125	29 (23)	1,123	232 (21)
Buffer	12	15	1 (7)	176	14 (10)

Numbers in parentheses are percentages.

1 Adult HSC were enriched for CD34 expression as outlined in methods. Fetal HSC were purified from fetal livers as outlined in methods.

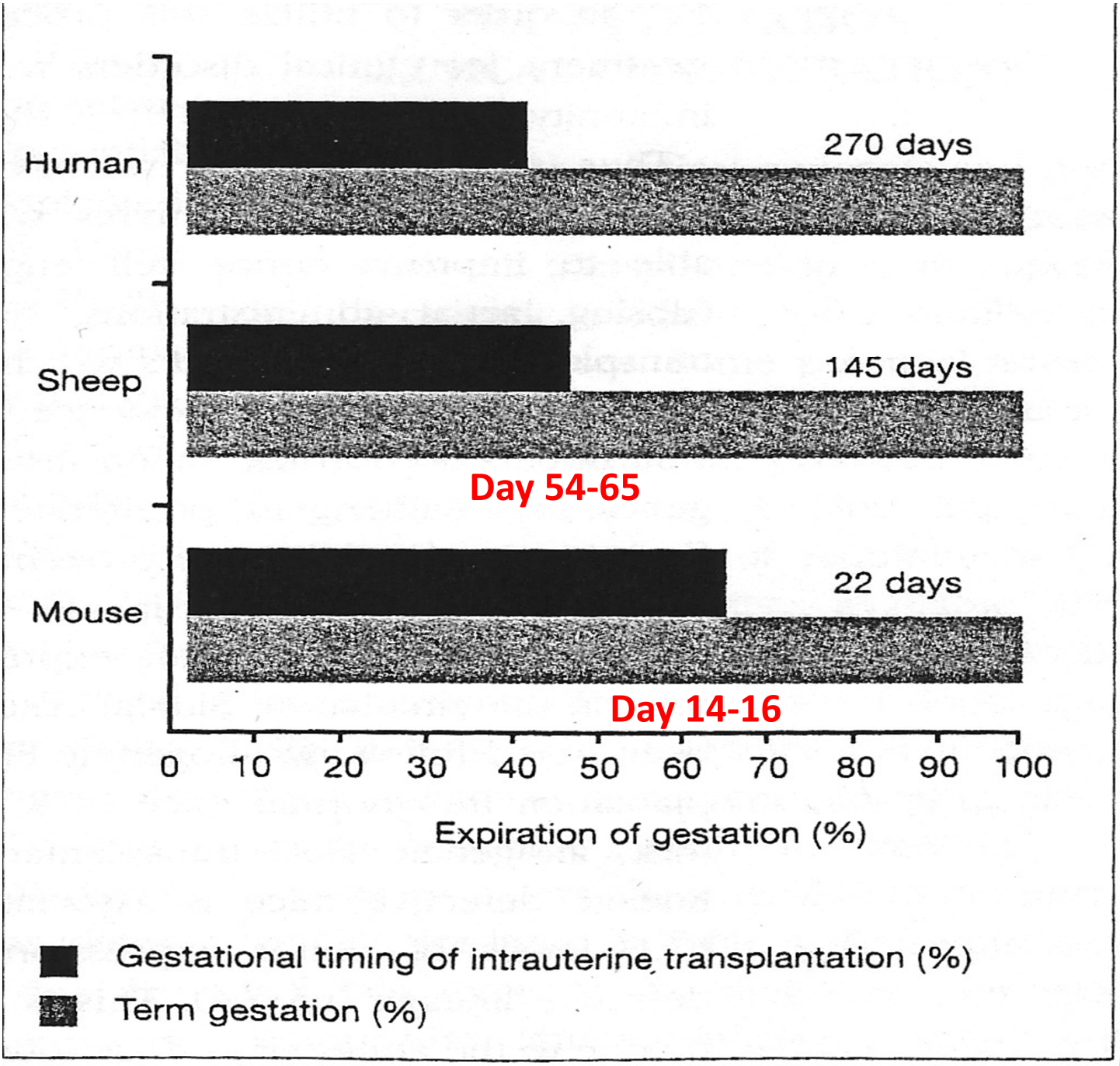
2 From mothers surviving surgery.

3 The difference in fetal survival following surgery on day 12 of gestation was not significantly different ($p = 0.148$) using adult as opposed to fetal HSC. The difference in fetal survival following transplantation of adult HSC after day 12 of gestation was significant ($p < 0.0001$, see text for details).

Engraftment frequency or chimeric index

Engraftment = marrow presence usually HSC phenotype
Expression = differentiation markers external to the marrow

Pathobiology 1998; 66: 230-39



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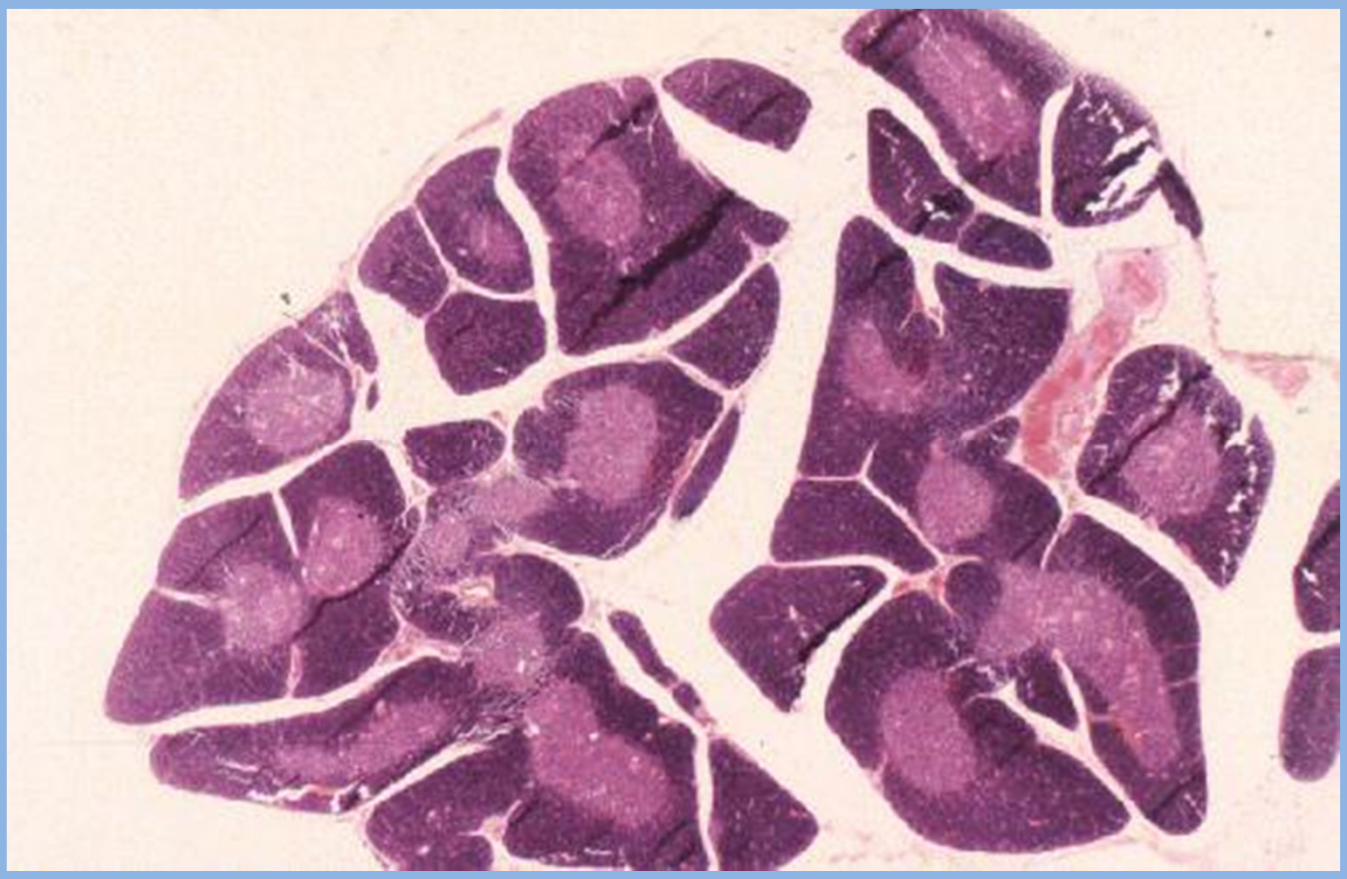
Reviews in Clinical and Experimental Hematology 8: 11-32, 1999



Embryology of the thymus:

At a defined stage in development the thymus demarcates and undergoes vascularization with formation of the medulla.

Gestational timing of demarcation:

Sheep beyond day 50

Mouse day 14-16

Immunology 1987; 62: 97–105.

Eur J Immunol 2000; 30:1948-1956 [PMID: 10940884]

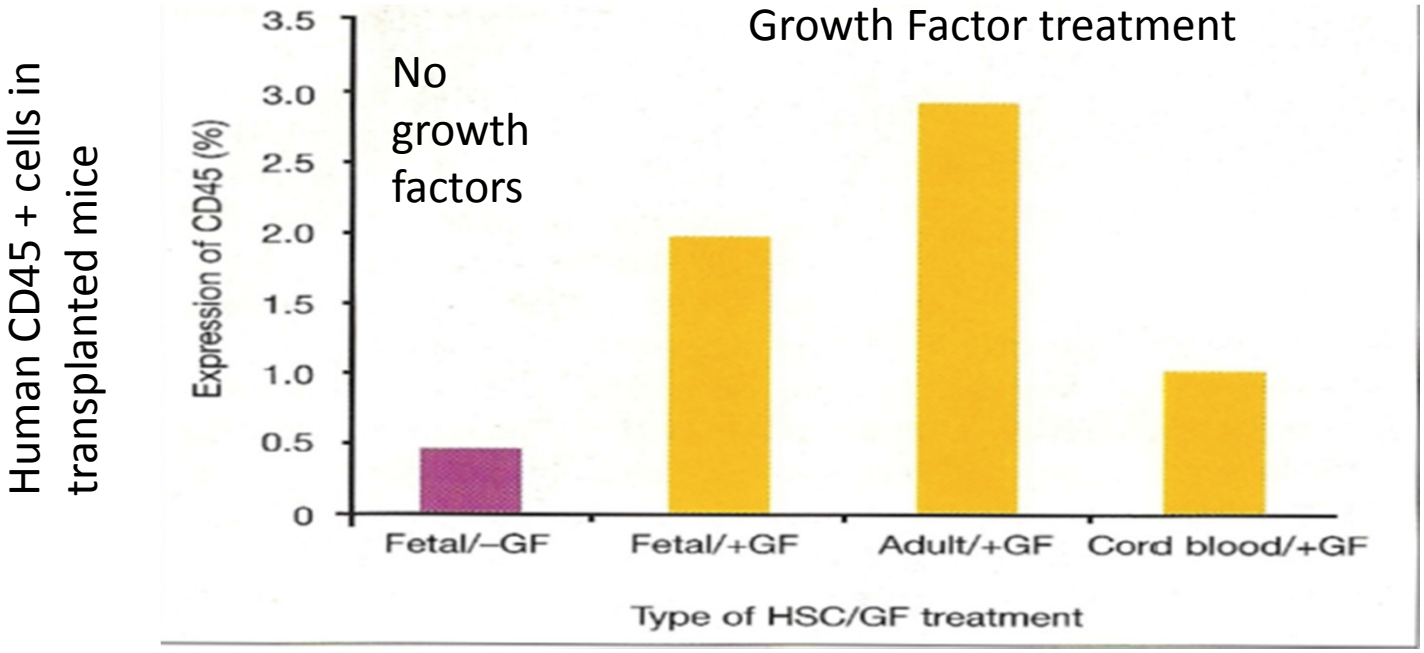
Table 2 Groups of 6 sheep were assessed for tolerance to allogeneic hematopoietic stem cells (HSC) and then re-transplanted after birth with same donor HSC

Allogeneic sheep HSC render recipient sheep[1] tolerant following in utero transplantation		
Stimulator	Responder	Stimulation index[2]
Donor	Donor	0
Recipient	Recipient	0
Donor	Recipient	0-8
Recipient	Donor	58 ± 11
Pooled	Donor	69 ± 12
Pooled	Recipient	78 ± 12
Postnatal infusion of allogeneic same donor HSC augments engraftment in tolerant sheep[3]		
% donor cells at birth	*n*	% increase[4]
6-10	4	86 ± 29
11-15	5	63 ± 22
> 15	4	21 ± 11

[1]Representative sample of 6 chimeric lambs; [2]Variation of mixed lymphocyte reaction (MLR) previously reported[32]; [3]Tolerance determined *via* MLR[32]; [4]Assessed 6 mo after postnatal stem cell infusion (3×10^8 cells/kg) in thirteen chimeric lambs rendered tolerant. HSC: Hematopoietic stem cells.

World J Stem Cells 2013 April 26; 5(2): 43-52
doi:10.4252/wjsc.v5.i2.43

Human (xeno) and allogeneic grafts are expandable after *in utero* transplantation



Allogeneic in mice

- Prenatal tolerance induction and postnatal nonmyeloablative bone marrow transplantation
  Blood. 2002; 100: 2225-2234

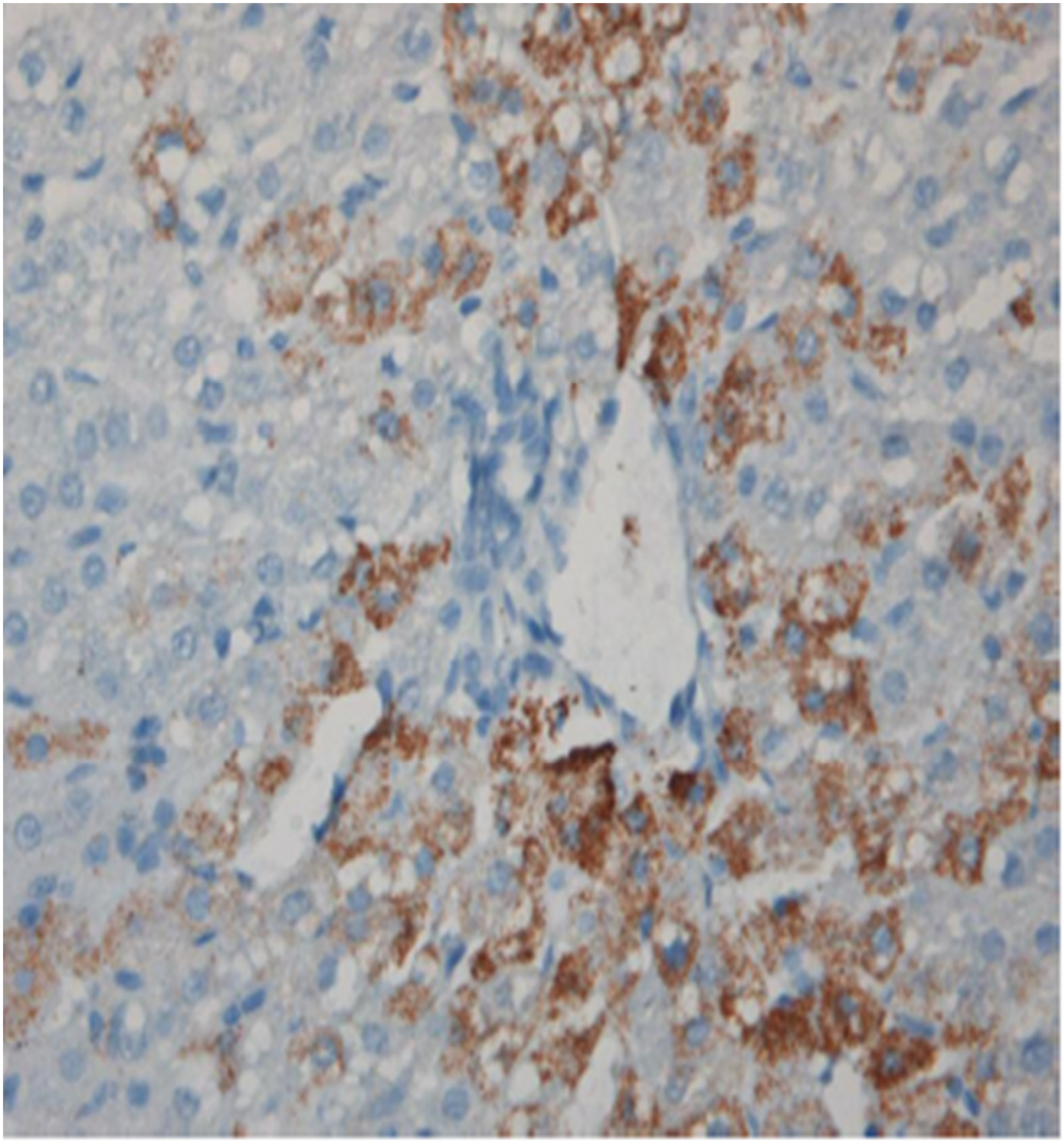
Xenogeneic (human) in sheep

- Postnatal administration of human growth factors
  Exp Hematol. 2007; 35: 1594–1600.

Table 1 Human proteins detected in circulation of animals transplanted in utero with human stem cells

Animal transplanted	Human protein detected
Sheep	IgM[1]
Sheep	Albumin[47,48]
Sheep	Factor VIII[1]
Sheep	C-peptide[49,50]
Sheep	α-fetoprotein[1]
Mouse	IgM[23]

World J Stem Cells 2013 April 26; 5(2): 43-52
doi:10.4252/wjsc.v5.i2.43



Liver section obtained at 11 months post transplant from sheep transplanted with CB-derived CD34-Lin- cells and stained with anti-human hepatocyte antibody

Tolerance to solid organ antigens/ Where are sheep NK cells?

Blood 2004; 104: 2582-2590
[PMID: 15231580 DOI: 10.1182/blood-2004-01-0259]

Stem Cell (SC) populations studied for in vivo differentiative capacity

1. Human fetal pancreas derived mesenchymal SC

   Exp Hematol 2010; 38: 311-320 [PMID: 20170708 DOI: 10.1016/j.exphem.2010.02.005]

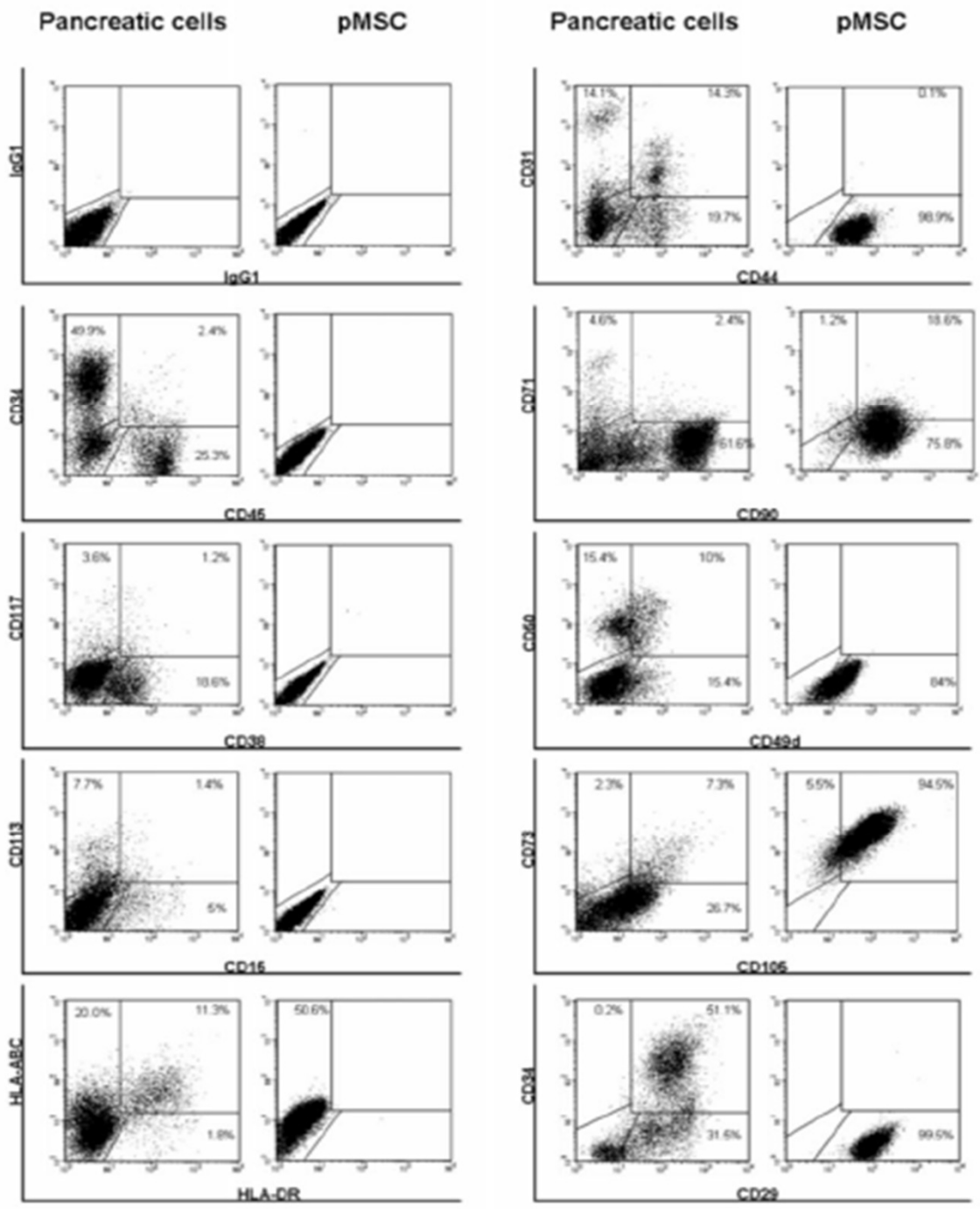
2. Embryonic SC derived CD34 cells

- using mouse S17 BM stromal cell line and

- embryoid body formation and differentiation *in vitro*

  Exp Hematol 2010; 38: 516-525.e4 [PMID: 20227460 DOI: 10.1016/j.exphem.2010.03.002]

hfpSC (human fetal pancreatic SC) phenotype in comparison to input population



hfpSC + for mesenchymal markers: CD29, 44, 73,90 and 105

Real time quantitative PCR detection of human DNA following transplantation with hfpSC (human fetal pancreatic stem cells)

Animal no.	When harvested (months after transplant)	% Human DNA			
2349	27	ND;	0.0001;	0.0004;	0.002
2351	27	ND;	ND;	ND;	ND
2352	27	ND;	ND;	ND;	0.0008
2353	25	ND;	ND;	ND;	ND
2354	7	ND;	0.0009;	0.0001;	ND
2356	27	ND;	0.0001;	ND;	0.0002
2358	25	ND;	ND;	0.002;	0.0002
2359	27	0.004;	ND;	ND;	ND
2360	27	0.001;	ND;	ND;	ND
2362	27	ND;	0.002;	ND;	ND

ND: Not detected, Limit of detection: $\geq$ 0.0001

- Four 1 mm^3 samples from the pancreatic tail
- Limited sampling method
- Chimeric incidence: 79%

Circulating human insulin in sheep 7 months to 2 years after transplantation of hfpSC *in utero*

Animal no.	When sampled (months post-transplant)	Human C-peptide (ng/mL)	CV%
2349	7	0.43	± 5%
	25	2.73	± 10%
	27	0.75	± 8%
2351	7	ND	
	25	ND	
	27	ND	
2352	7	0.47	± 1%
	25	0.36	± 9%
	27	0.32	± 1%
2353	7	ND	
	25	ND	
2354	7	ND	
2356	7	1.86	± 14%
	25	1.46	± 6%
	27	0.83	± 4%
2358	7	ND	
	25	ND	
2359	7	ND	
	25	ND	
	27	ND	
2360	7	1.21	± 1%
	25	4.49	± 2%
	27	ND	
2362	7	0.76	± 6%
	25	0.56	± 5%
	27	ND	

Exp Hematol 2010; 38: 311-320 [PMID: 20170708 DOI: 10.1016/j.exphem.2010.02.005]

Human C-peptide levels in sheep ~ 2-3 years after transplantation of human ESD-CD34+ SC during the fetal tolerance window

Animal no.	Cells transplanted	When sampled (months posttransplantation	Human C-peptide (ng/mL)[a] ± CV (%)[b]	Glucose (mM) ± CV (%)[b]
1748	140,000	38	0.72 ± 8	3.3 ± 1
	$CD34^+/CD38^-$ on S17 (day 17)	39[c]	0.77 ± 7	2.4 ± 1
		39[c]	0.38 ± 10	2.8 ± 6
1876[d]	23,500	27	0.75 ± 1	3.6 ± 4
	$CD34^+/Lin^-$ on S17 (day 17)	29	<0.32[e] ± 14	3.4 ± 1
1885	63,000	28	1.52 ± 5	2.8 ± 3
	$CD34^+/Lin^-$ on S17 (day 17)	31	1.35 ± 3	3.6 ± 1
		32	0.62 ± 10	3.5 ± 0
1887	70,000	29	0.59 ± 3	2.5 ± 6
	$CD34^+/Lin^-$ on S17 (day 17)	31	0.66 ± 3	2.6 ± 1
		55	0.77 ± 5	2.9 ± 4
1939	1,000,000	31	3.53 ± 1	2.8 ± 3
	All cells from S17 (day 17)	33	1.40 ± 5	2.9 ± 1
		52	2.30 ± 8	2.8 ± 5
1952	1,000,000	24	1.79 ± 2	ND
	All cells from S17 (day 18)	29	4.04 ± 4	3.0 ± 1
		30	1.23 ± 9	3.1 ± 4
2034	80,000	20	1.01 ± 9	ND
	$CD34^+/CD38^-$ on S17 (day 8)	23	1.96 ± 8	2.9 ± 1
		31	0.85 ± 15	2.5 ± 6
2135	100,000	20	1.21 ± 1	3.2 ± 2
	$CD34^+/CD38^-$ from EB (day 8)	23	4.49 ± 2	2.1 ± 2
		39	2.62 ± 6	2.1 ± 5

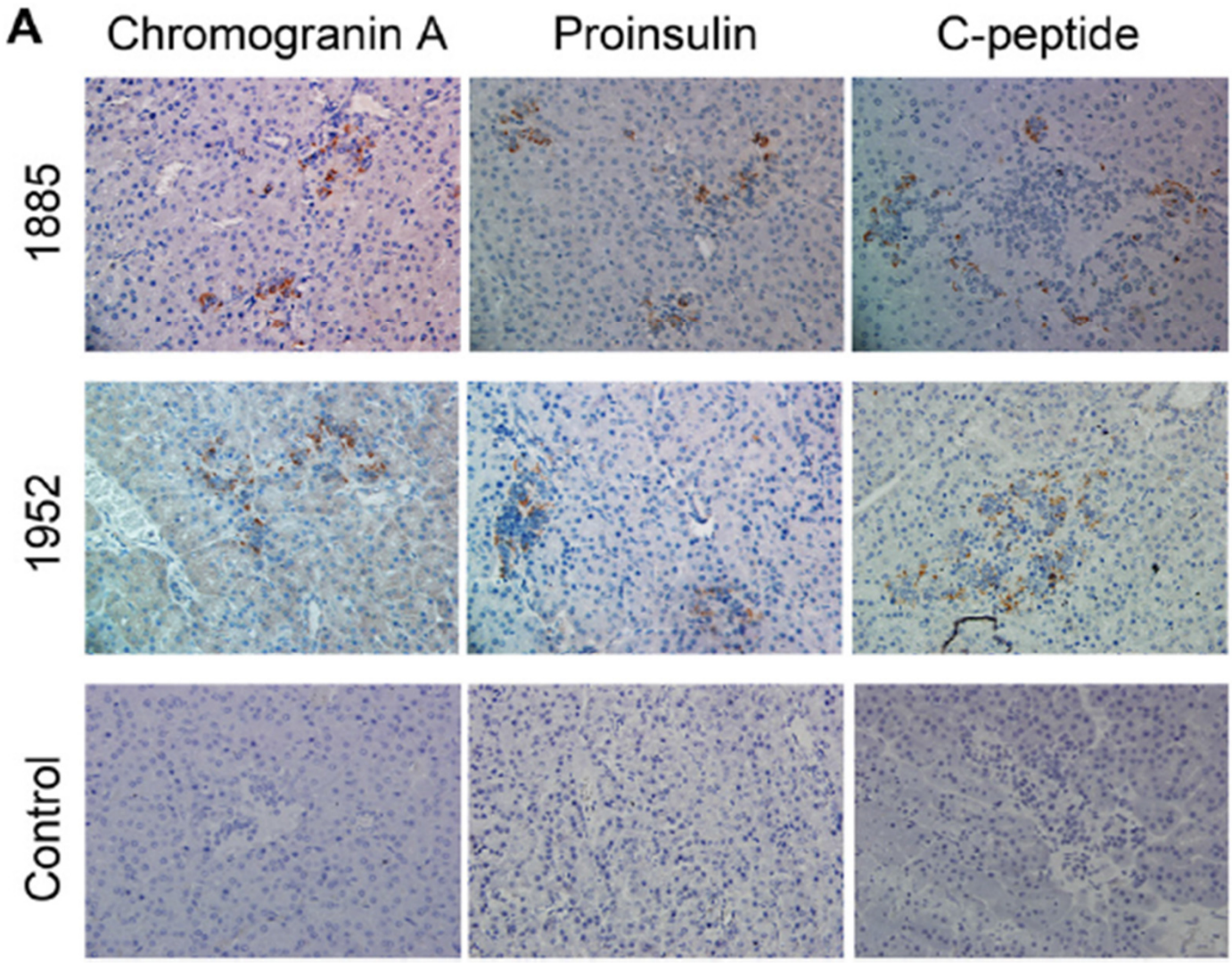
Twenty-three transplanted animals and 10 controls (nontransplanted) were sampled after fasting for 24 hours. Of these, eight transplanted animals had detectable levels of human C-peptide as reported here.

EB = embryoid body; ND = not done.

[a]Human C-peptide assay (enzyme-linked immunosorbent assay [ELISA]) standards ranged from 0.5 to 20 ng/mL with a detection limit of 0.32 ng/mL.

A.D. Goodrich et al. Exp Hematol 2010; 38: 516-525.e4 [PMID: 20227460 DOI: 10.1016/j.exphem.2010.03.002]

In situ human islet activity in 2 sheep transplanted with human stem cells



Chromogranin A is predominantly in α-cells

A.D. Goodrich et al. Exp Hematol 2010; 38: 516-525.e4
[PMID: 20227460 DOI: 10.1016/j.exphem.2010.03.002]

Summary and conclusion

1. Transplantation of allogeneic or xenogeneic SCs during the fetal tolerance window in any experimental animal renders that animal tolerant to donor specific SCs and their differentiated progeny including species specific proteins

2. Any mulitpotent self-renewing SC population would likely be effective

3. The (self-)tolerant environment allows unfettered differentiation into both hematopoietic and solid organ lineages

4. Present evidence suggests these grafts are expandable

Summary and conclusion continued

5. In utero transplantation is a powerful but poorly understood investigative method that:

- should provide detailed understanding of mechanisms underlying self-tolerance
- may provide alternative methods for the investigation of biologic systems
- may allow alternative solutions to a number of clinical problems

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