

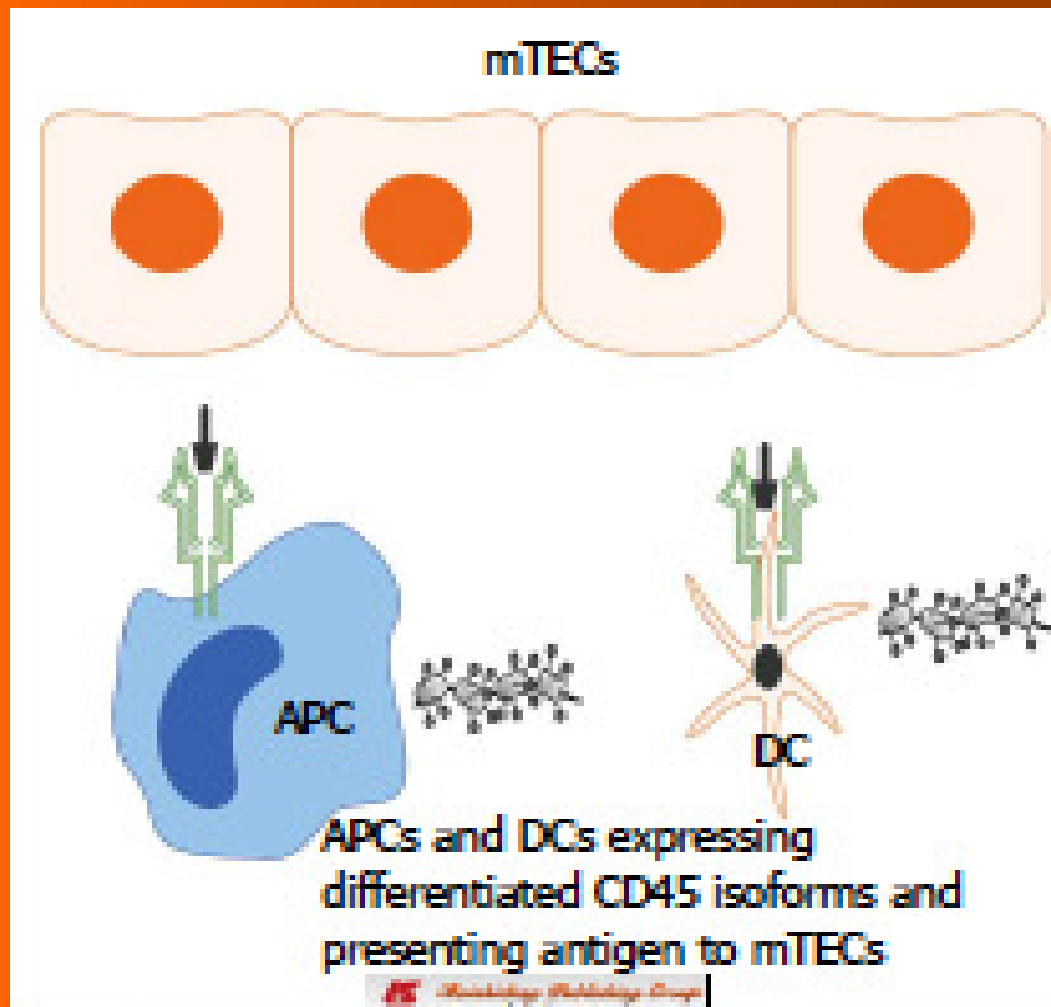
In utero transplantation:
(IUT) an experimental
window into immune self
Basic and applied implications

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World Journal of *Stem Cells*

World J Stem Cells 2009; April 26; 3(3): 49-60



“... 1955–1965 [was] the decade marked by the question, ‘Does autoimmunity exist?’ ...”

N. R. Rose and I R. Mackay



**Karl Landsteiner (1868–1943) in his Vienna laboratory in the early 1900s.
(Courtesy University of Wisconsin Middleton Library.)**

Nature Immunology 2001, 2: 279-281.

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

'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

By DR. R. E. BILLINGHAM*, L. BRENT and PROF. P. B. MEDAWAR, F.R.S.

Department of Zoology, University College, University of London

The inoculum from A-line male suspension in ringer's solution of small organized tissue clumps, isolated cells and cell debris prepared 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

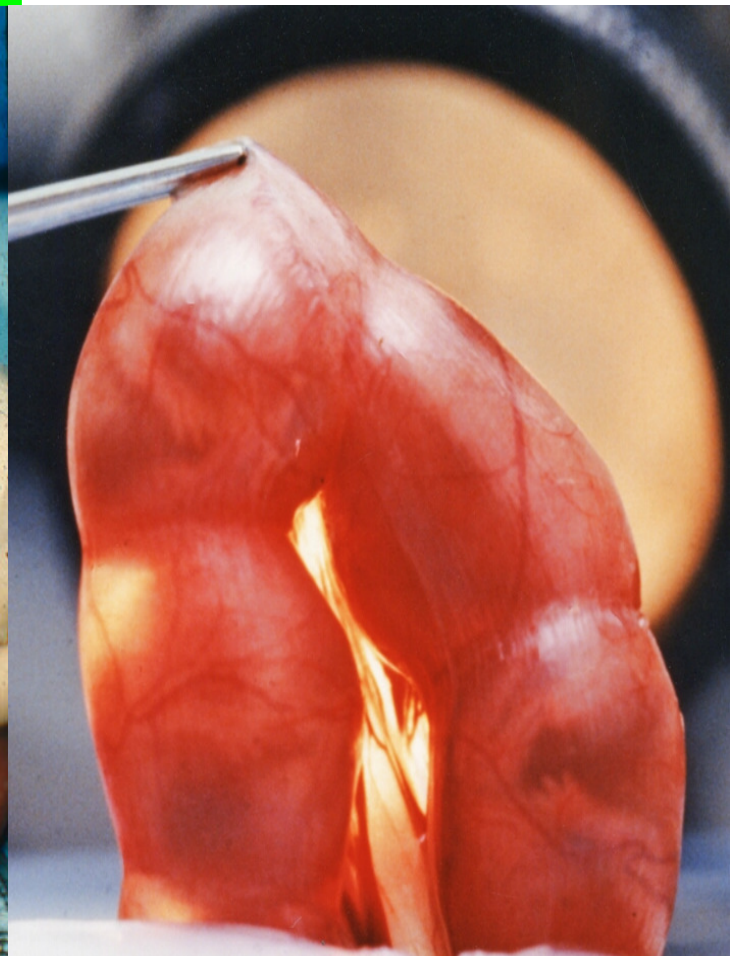
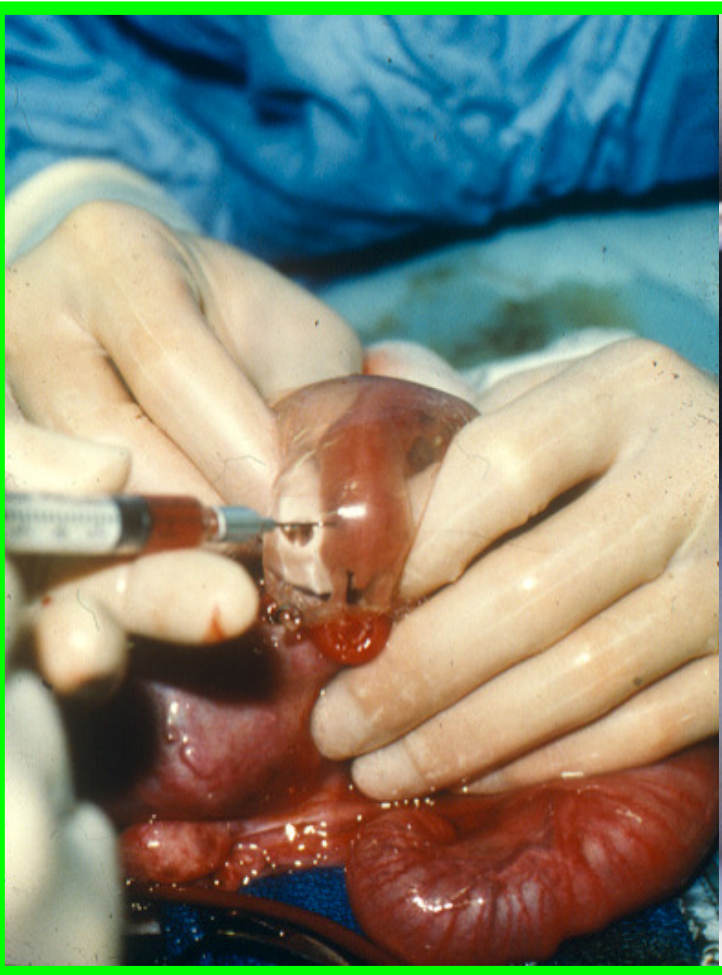
Summary

- 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*

*IP injection
on day 14-16*



SHEEP

MOUSE

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

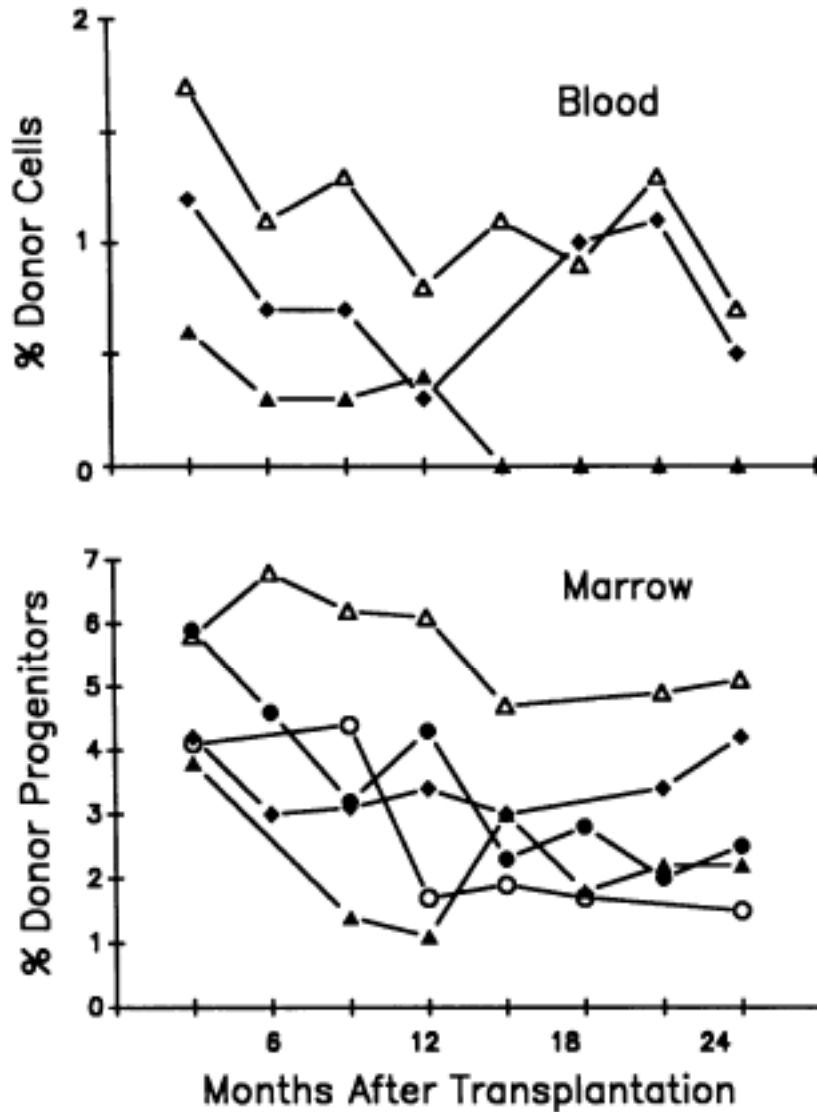
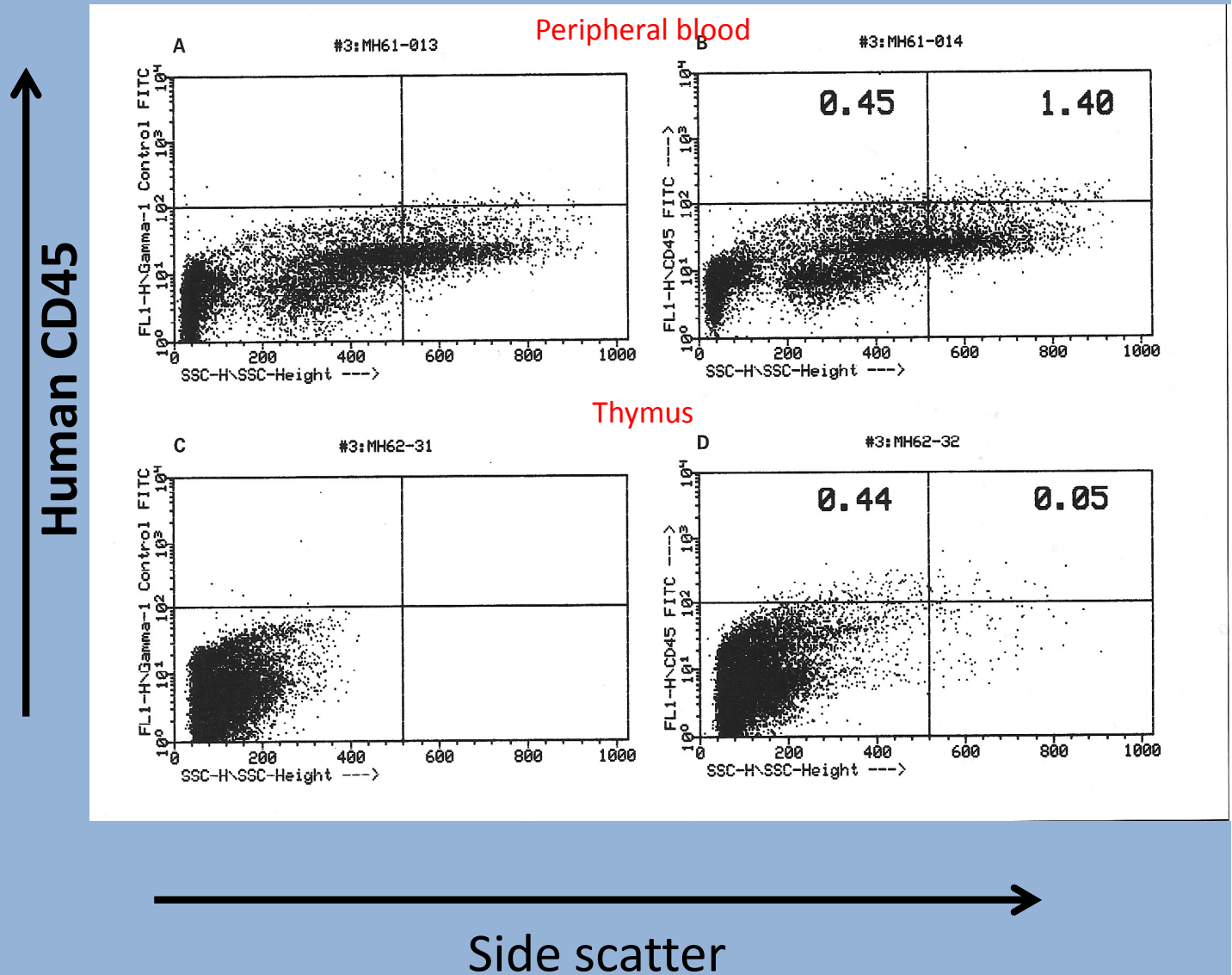
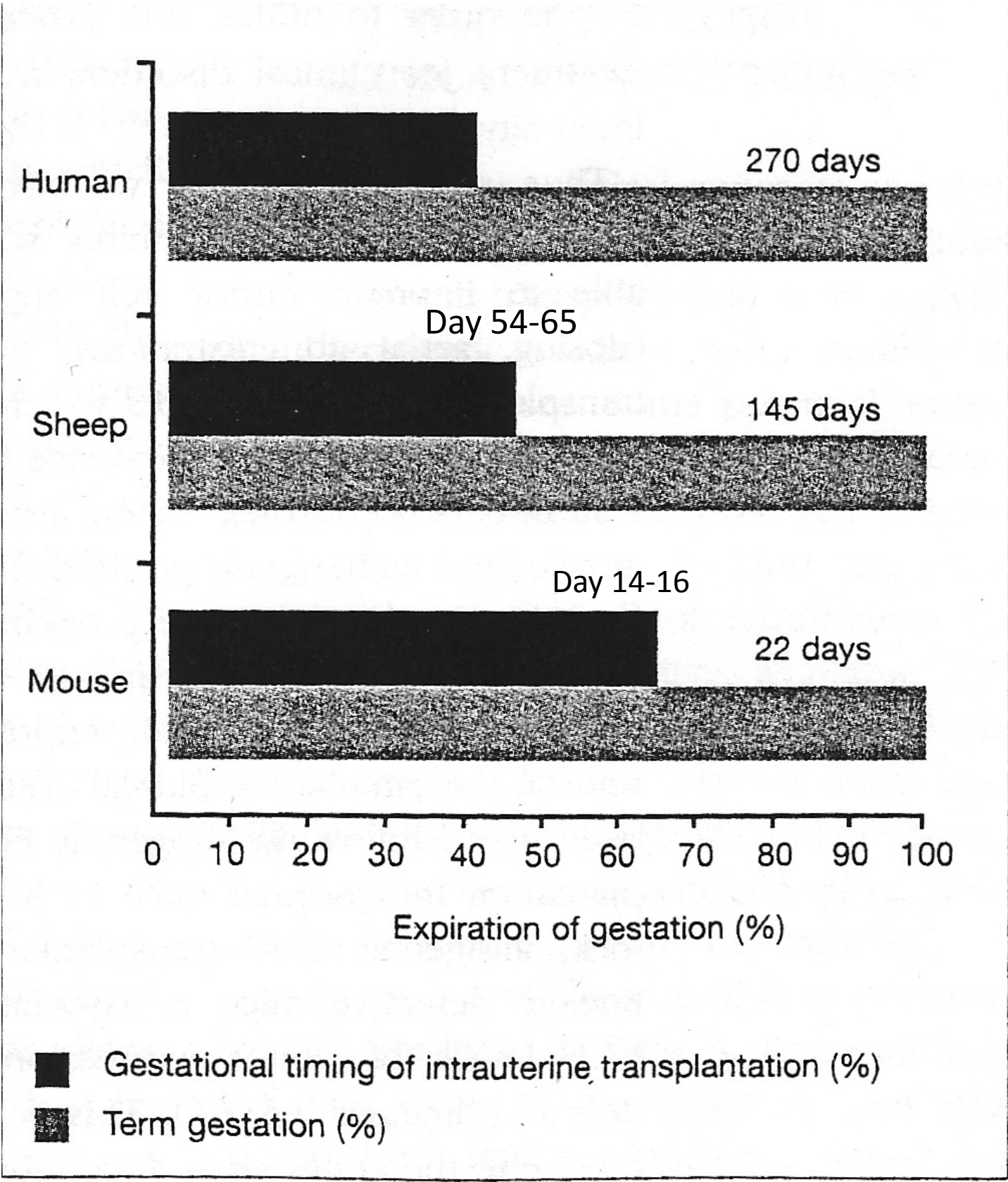


Figure 6. Persistence of donor (human) cells (blood)/progenitors (marrow) in chimeric sheep. Peripheral blood data are provided for the three animals that showed donor cells in circulation at birth. Bone marrow progenitors were assessed as described in text and legends to Fig. 3. ● — ●, 1219C; ○ — ○,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

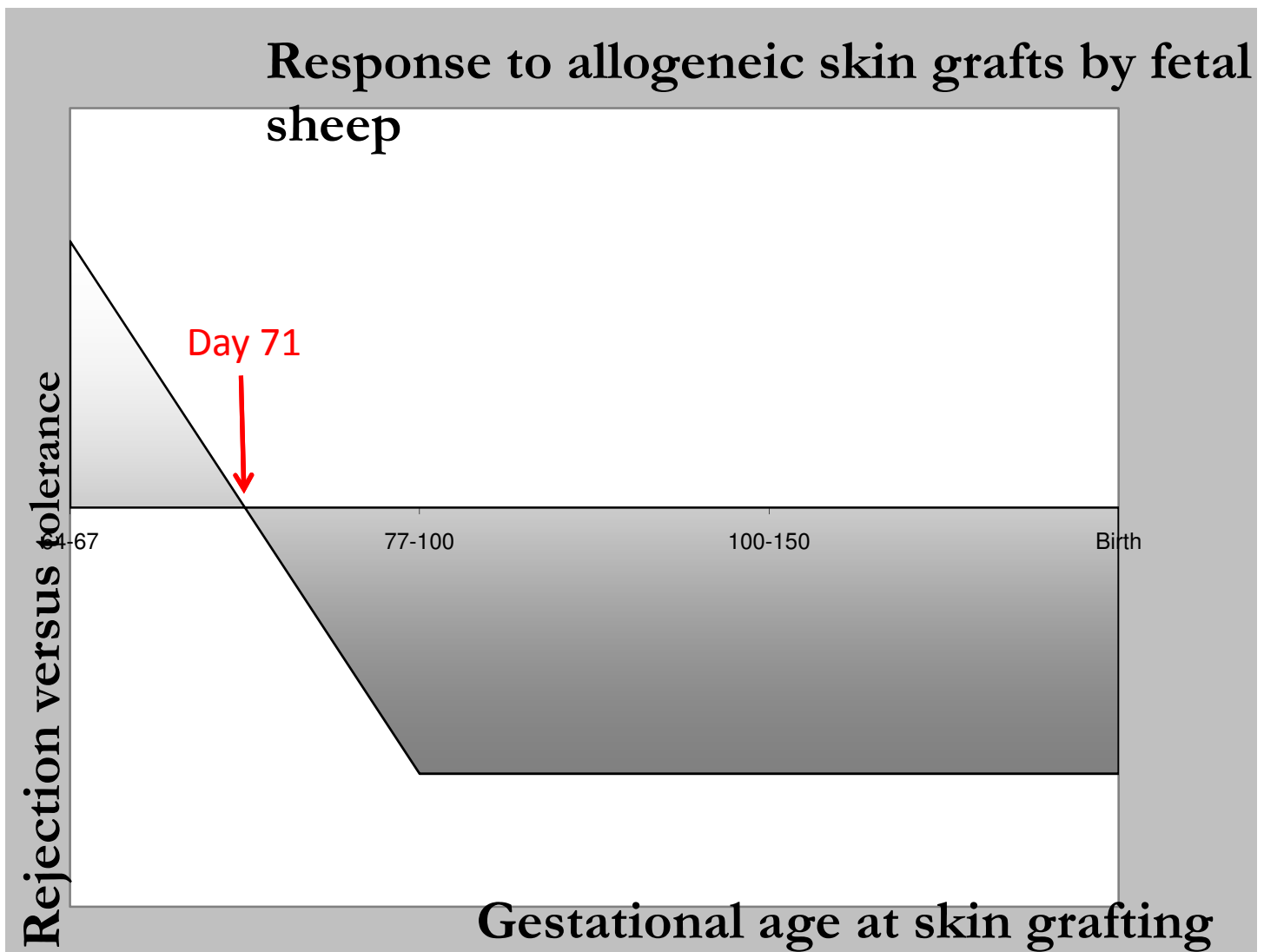
Fetal age at transplantation day 48-54 term gestation 145 days

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

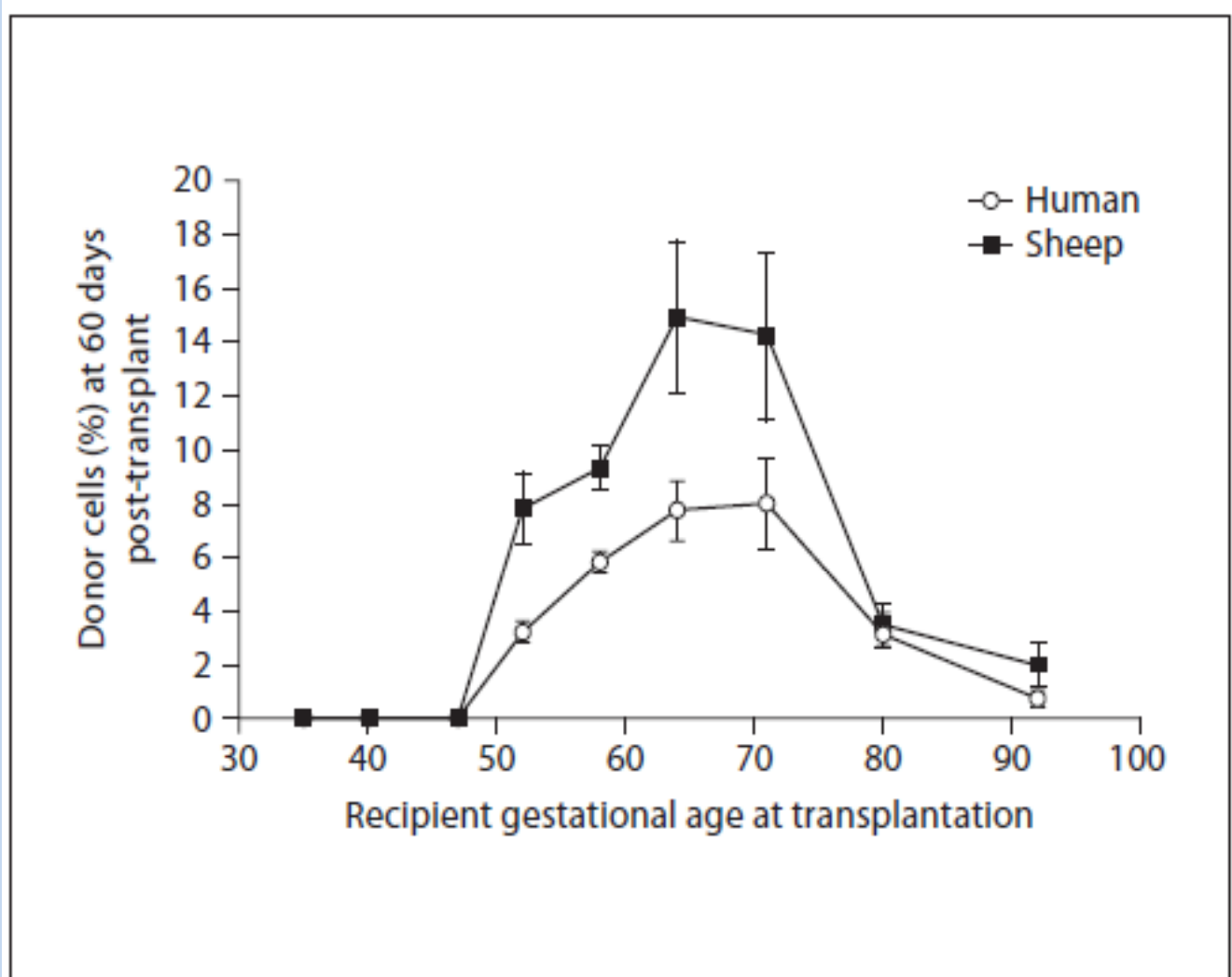




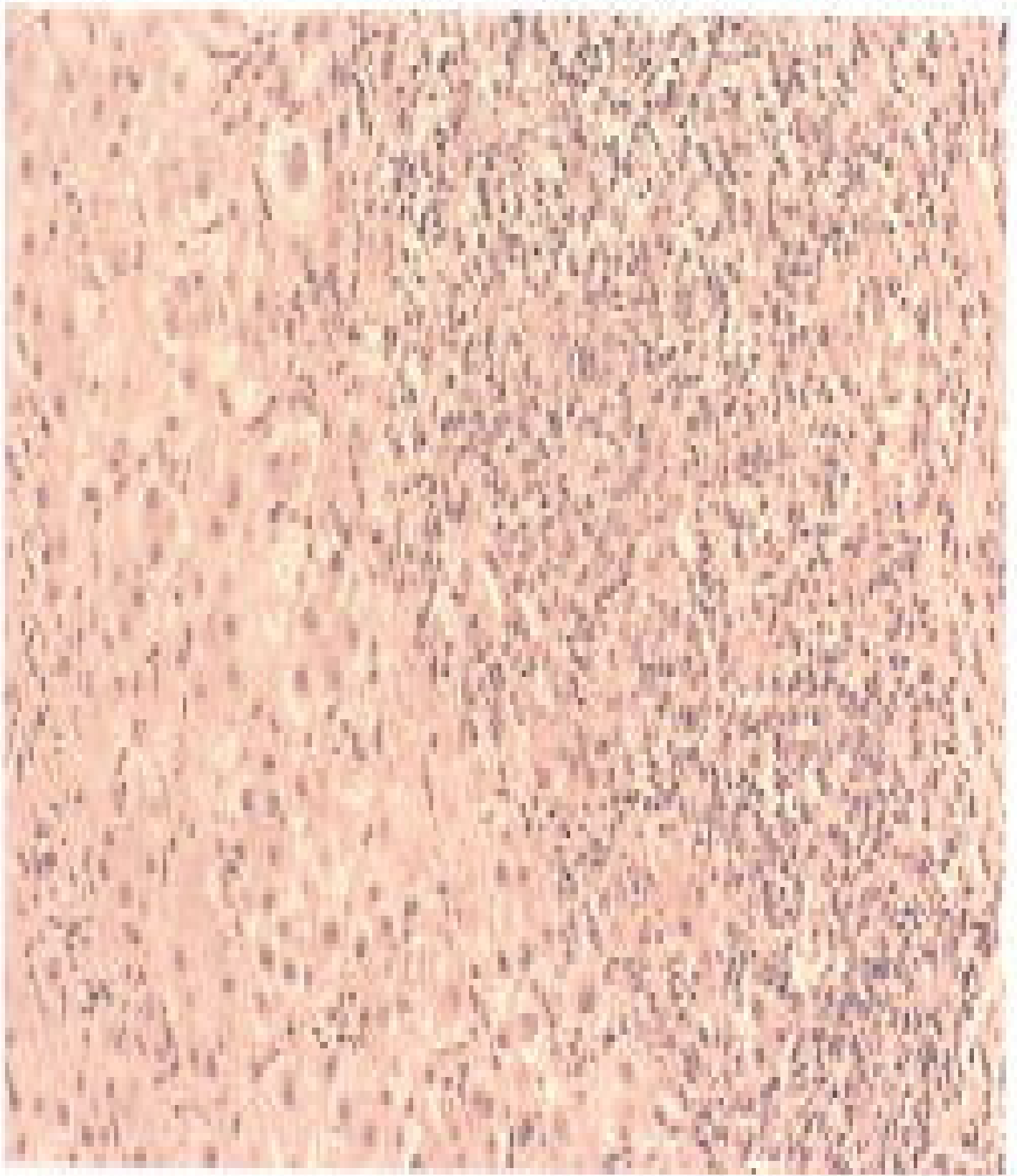
Silverstein A, Prendergast RA, Kraner K: Fetal response to antigenic stimulus. IV. Rejection of skin homografts by the fetal lamb. *J Exp Med* 1964; 119: 955–964.



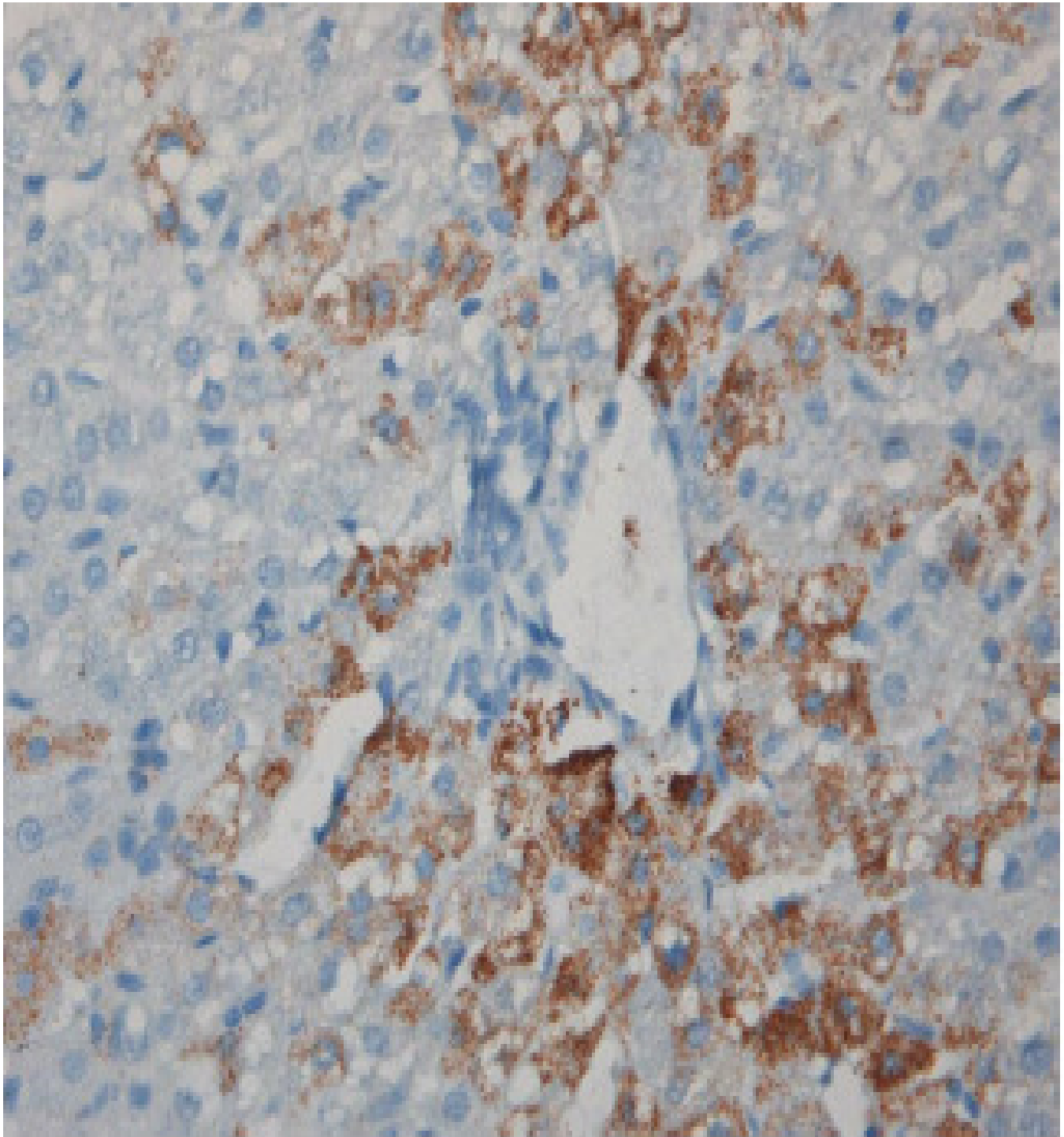
Engraftment receptivity is gestational age dependent



ENGRAFTMENT WINDOW: day 52-72



Interface hepatitis. The limiting plate of the portal tract is disrupted by a lymphoplasmacytic infiltrate. This histologic pattern is the hallmark of autoimmune hepatitis, but it is not disease specific. Hematoxylin and eosin, $\times 200$.



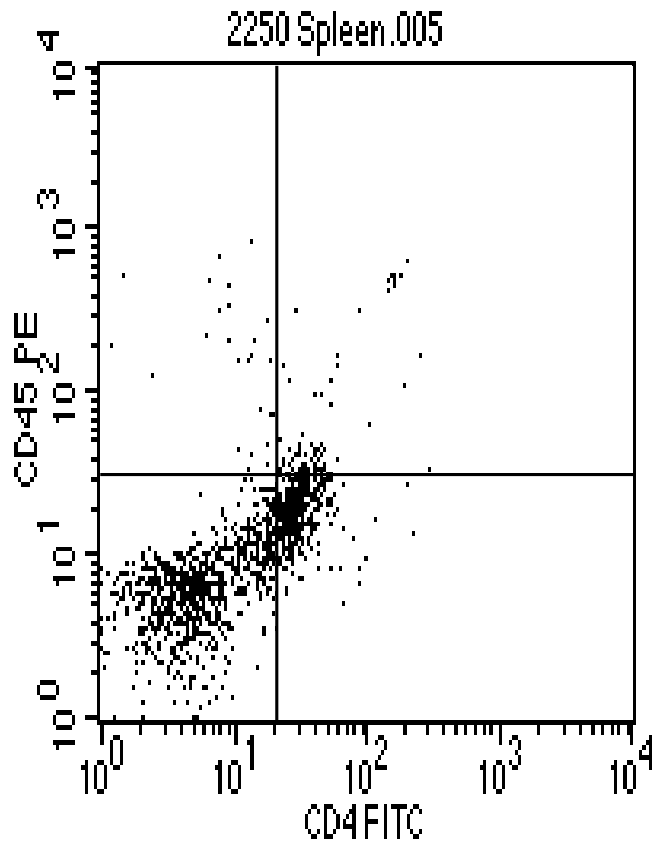
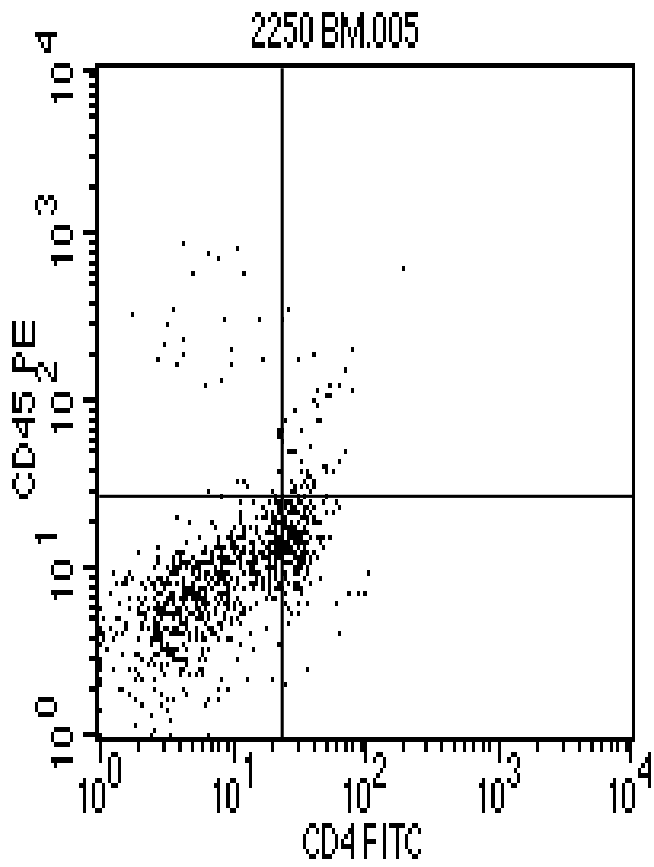
Liver section obtained at 11 months posttransplant from sheep transplanted with CB-derived CD34-Lin- cells and stained with anti-human hepatocyte antibody

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

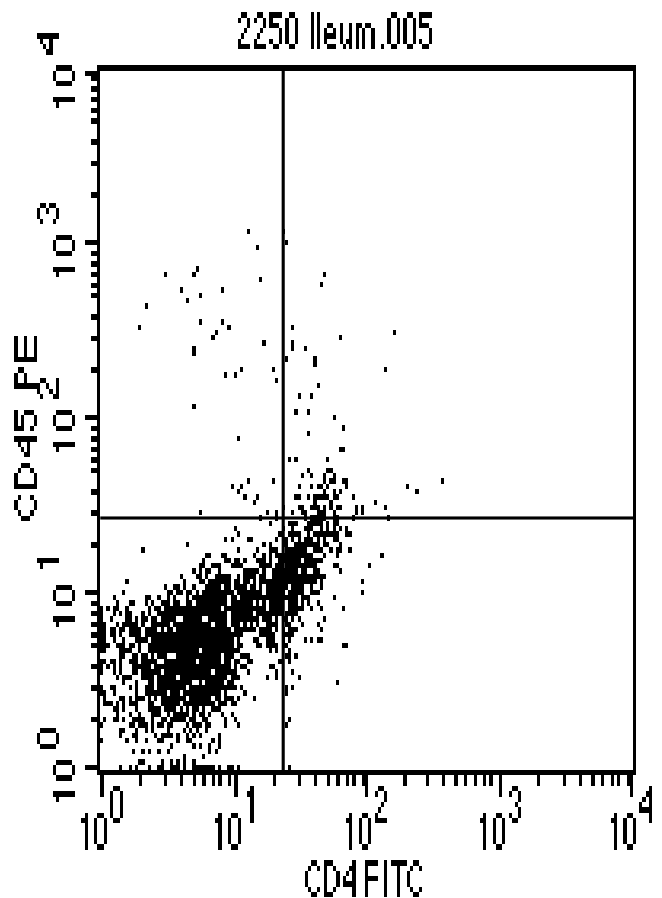
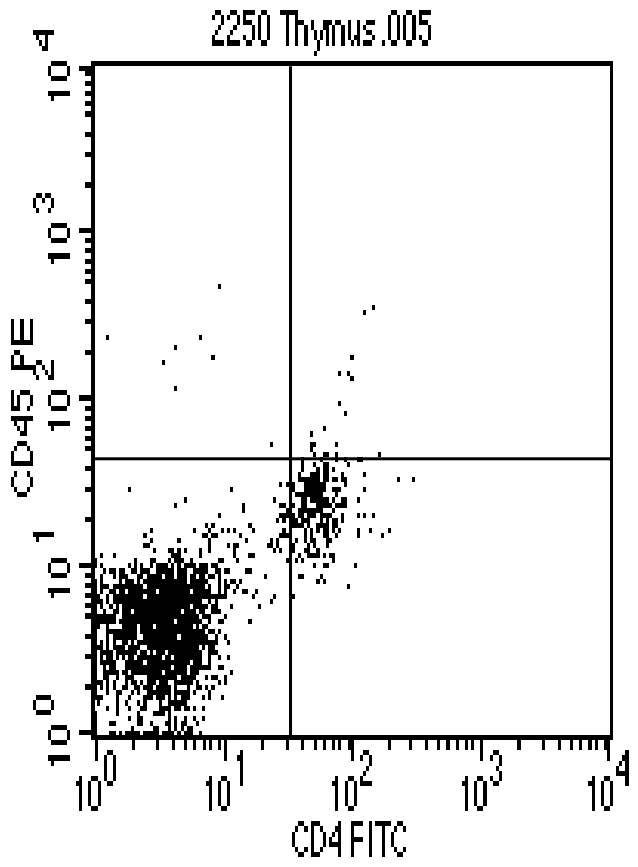
Blood 2004; 104: 2582-2590

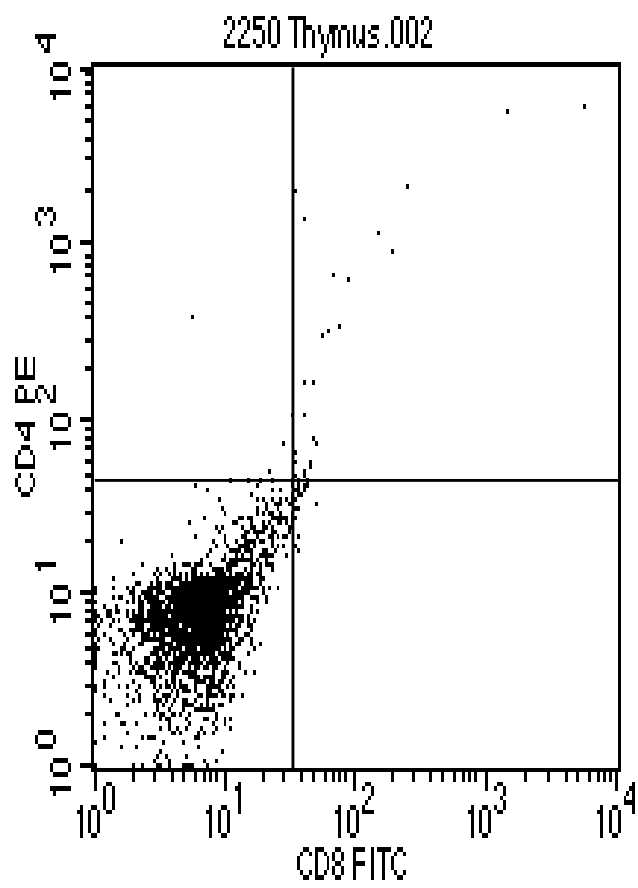
[PMID: 15231580 DOI: 10.1182/blood-2004-01-0259]

Phenotypic study of sheep lymphocytes at
varying gestational ages
Critical time day 45-72

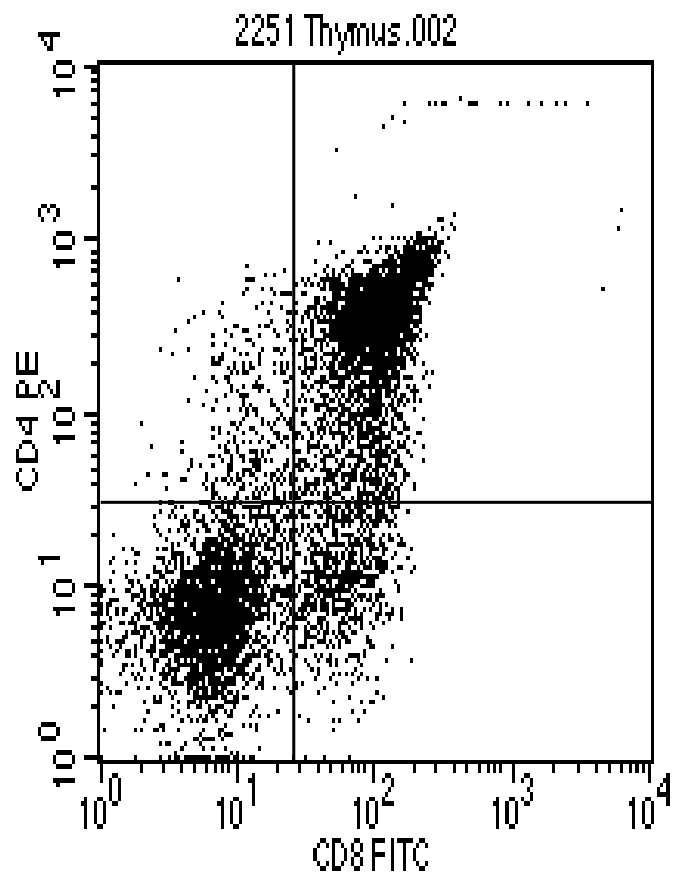


d39

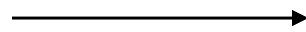


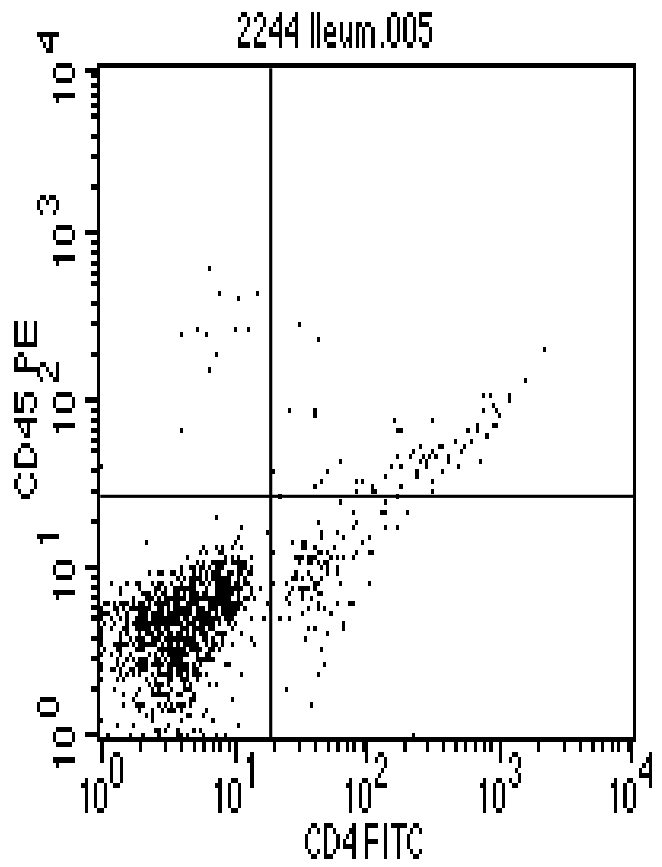
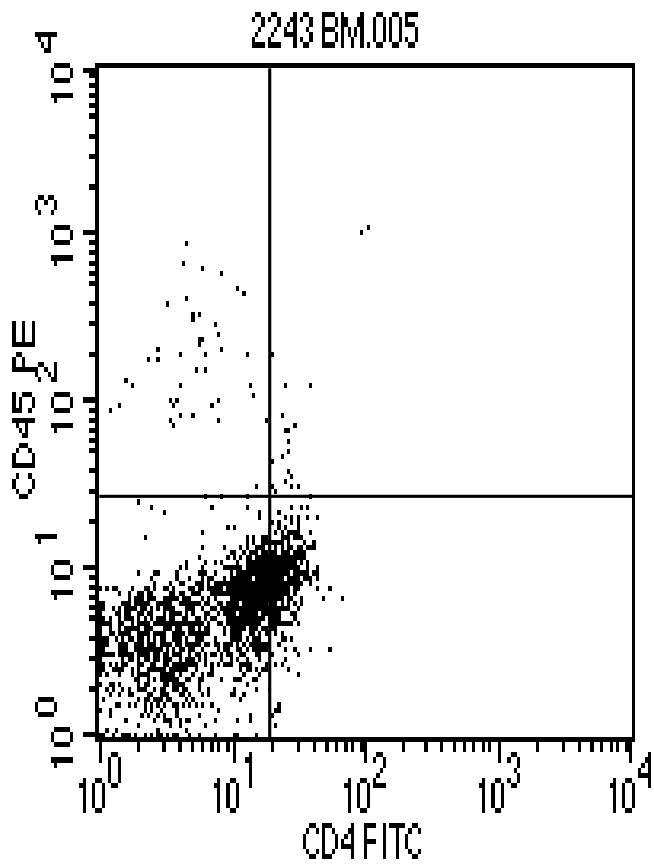


DAY 39

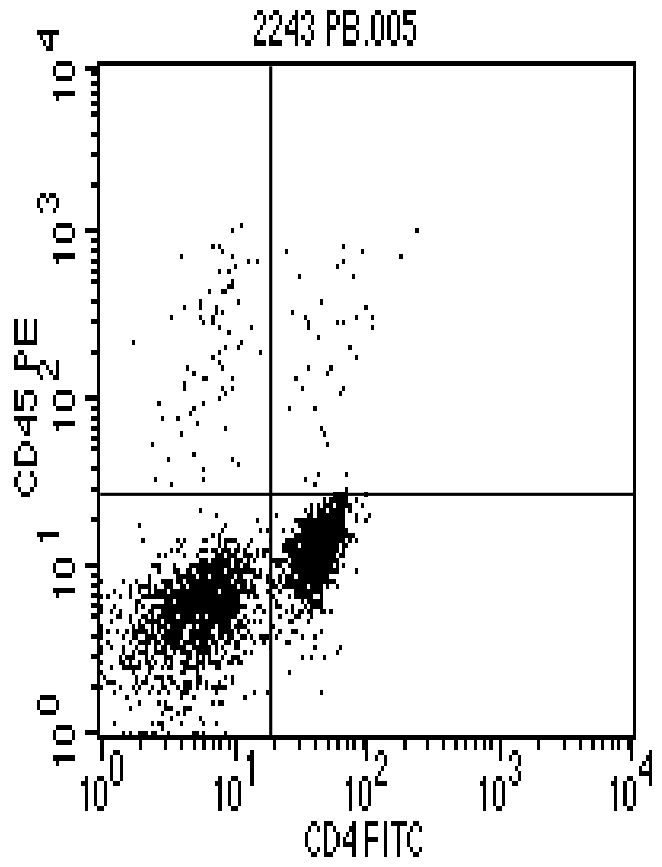
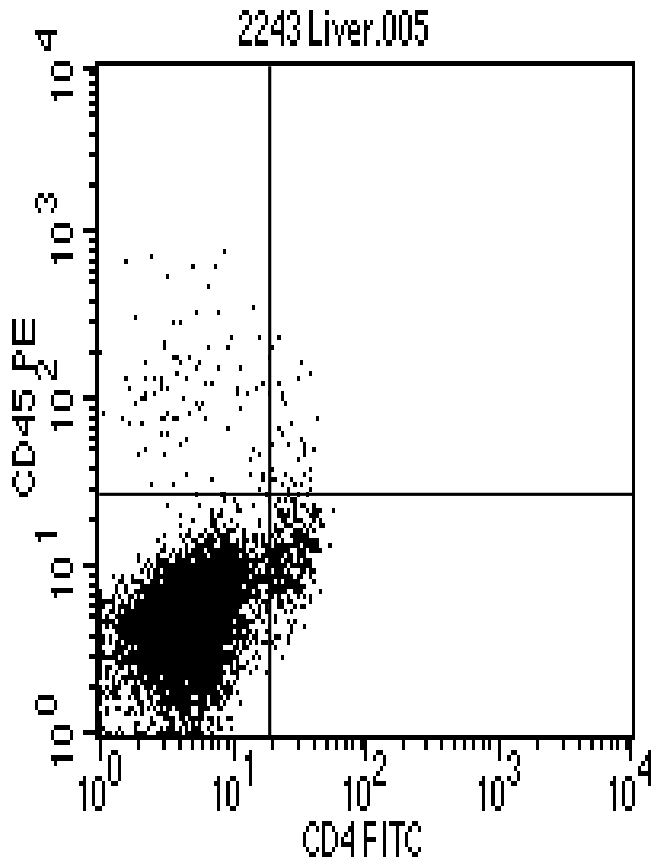


DAY 45

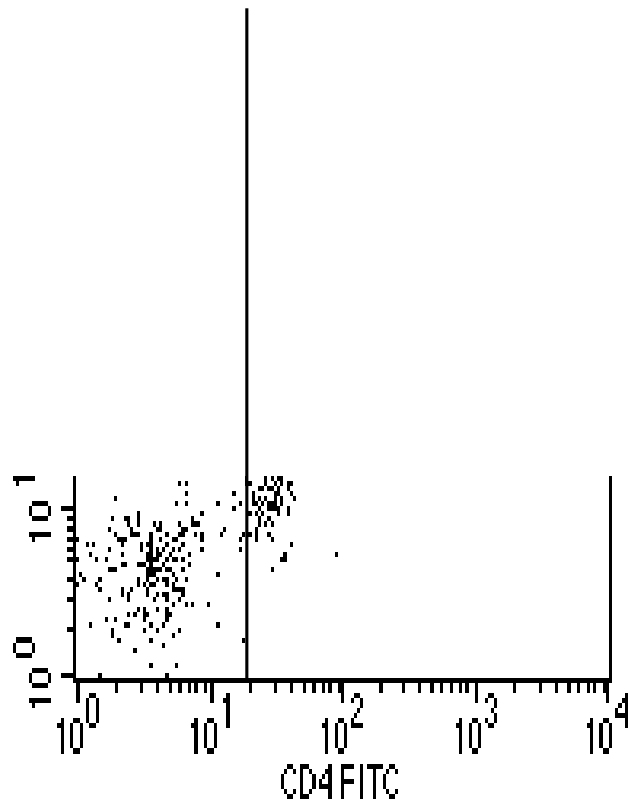




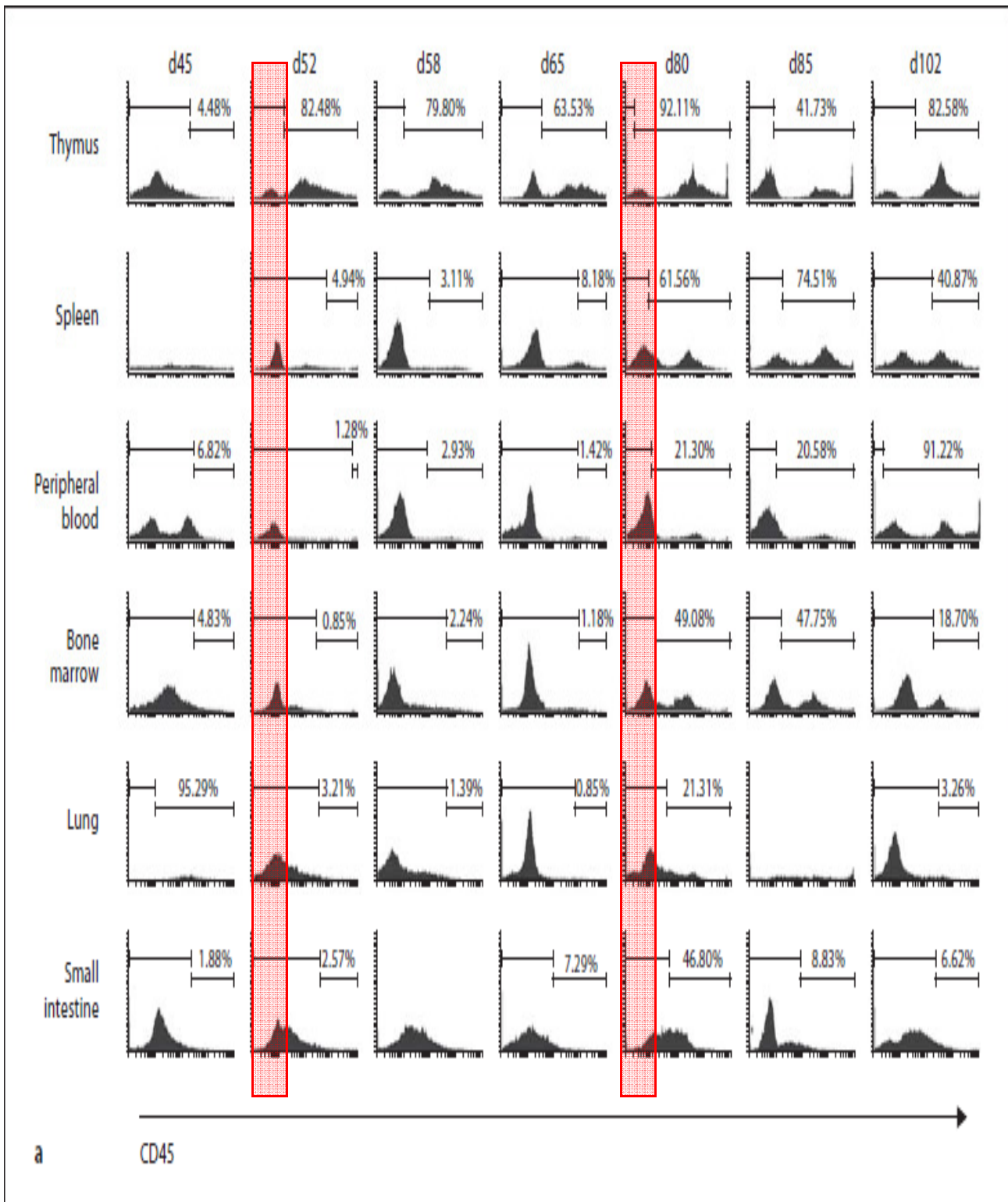
d58



d58

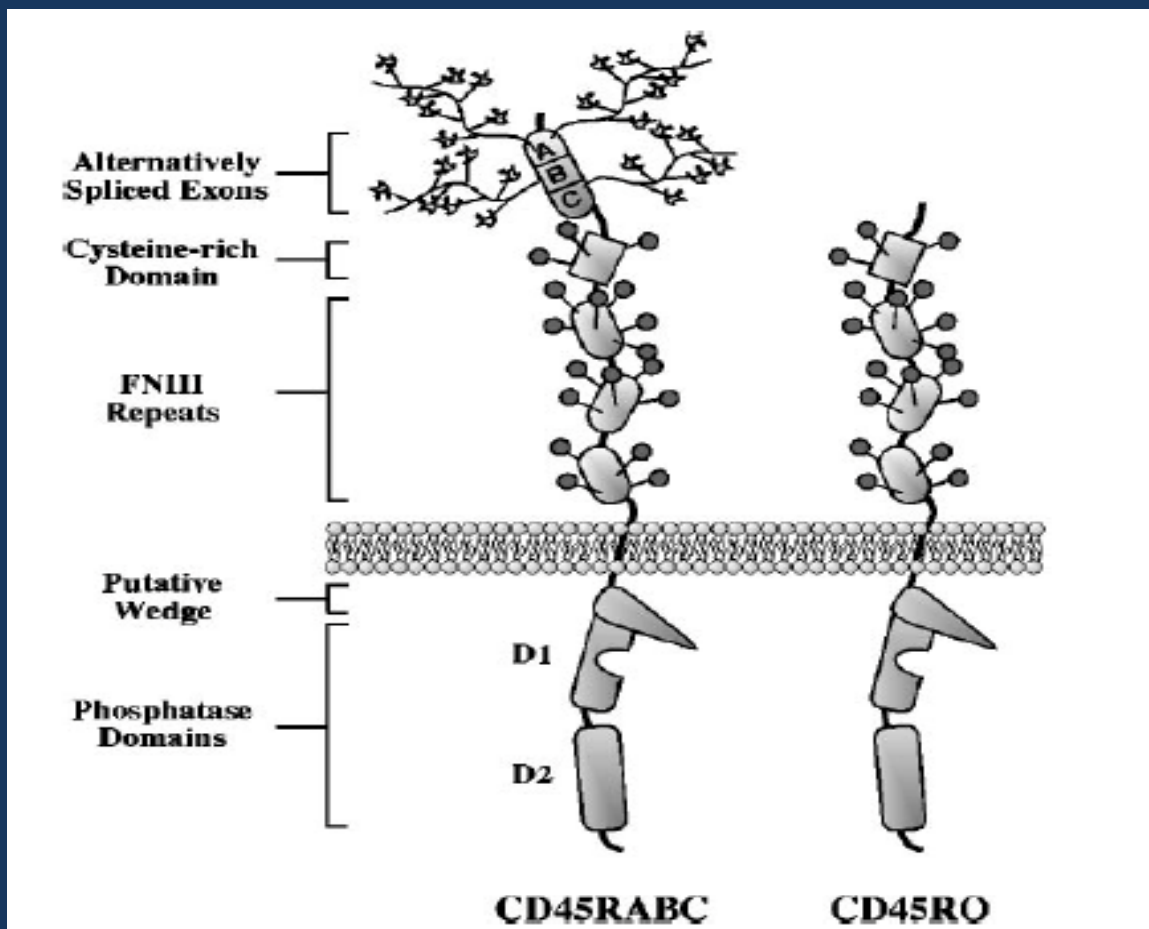


Antibody to sheep CD45 recognizes isoforms:
MW 225-190 Kilodaltons



a Representative histograms showing percent expression of CD45 on fetal ovine thymus, PB, spleen, bone marrow, lung, and small intestine at selected time points throughout gestation.

Fetal Diagn Ther 2009;25:102-110



- CD45 one of the most abundant cell surface glycoproteins (10% of cell surface) expressed on nucleated hematopoietic cells
- RABC (240 kDa -----> RO 180 kDa)
- Protein tyrosine phosphatase
- Controls the relative threshold sensitivity to external stimuli
- Aberrancy in CD45 function may lead to autoimmunity, immunodeficiency and malignancy
- Thymic selection generates T cells expressing self-reactive TCRs in the absence of CD45. *J Immunol* 2000; 165: 3073–3079.

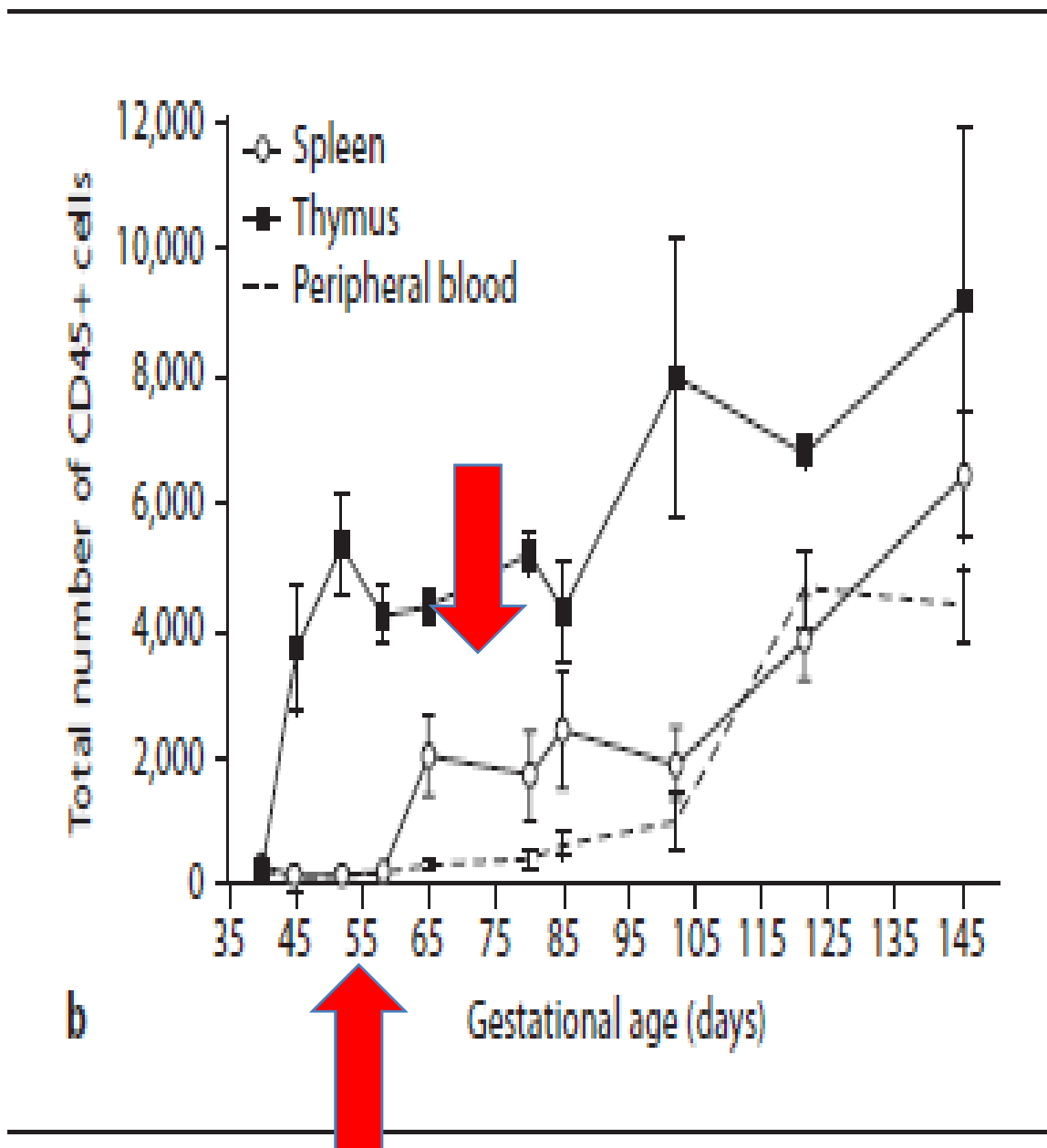
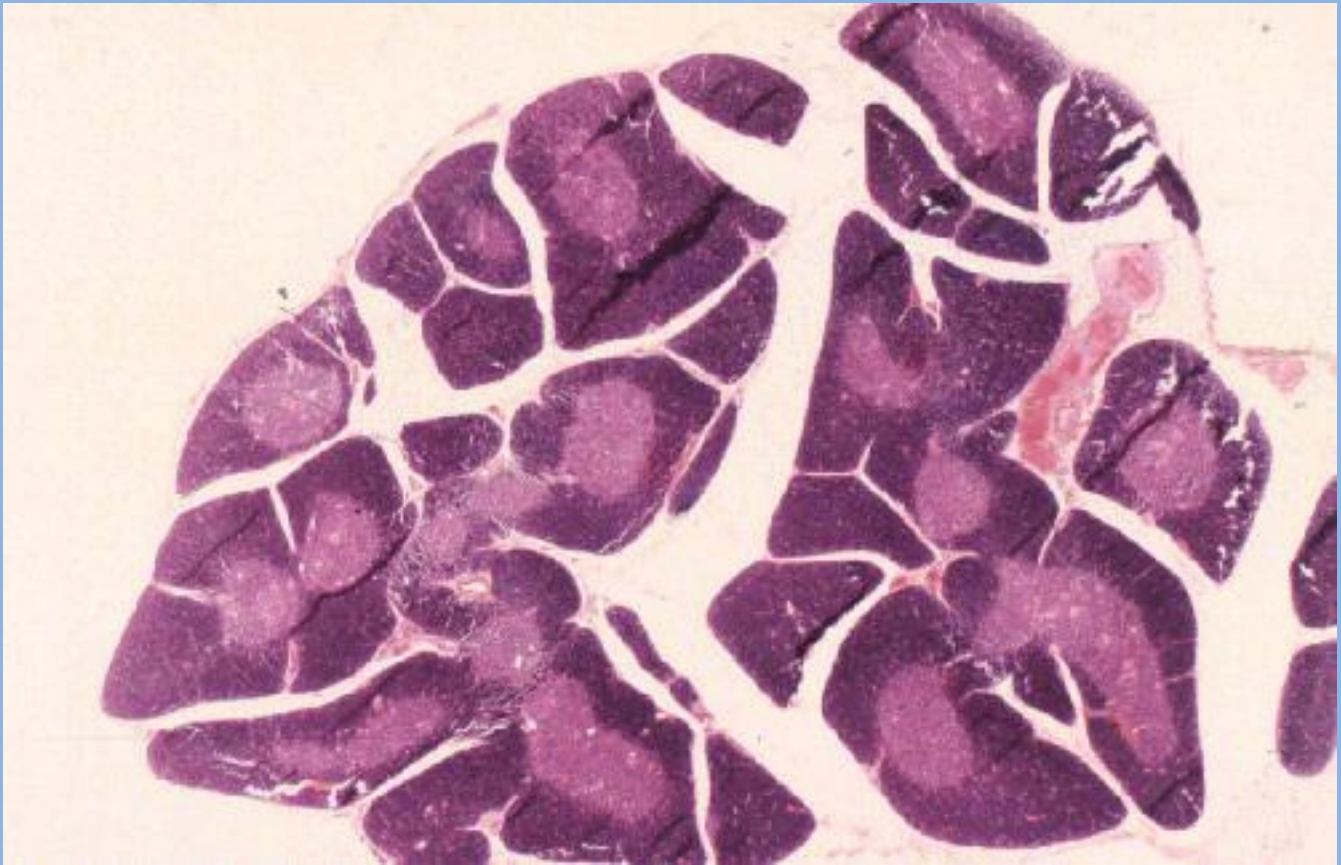


Figure 1. Expression of identifiable CD45 isoforms (225-190 kilodaltons) on cells of thymus and peripheral organs during sheep gestation.

b Cumulative data demonstrating expression of CD45 on cells from fetal ovine thymus, spleen, and PB throughout gestation. SEM is shown as error bars.



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:

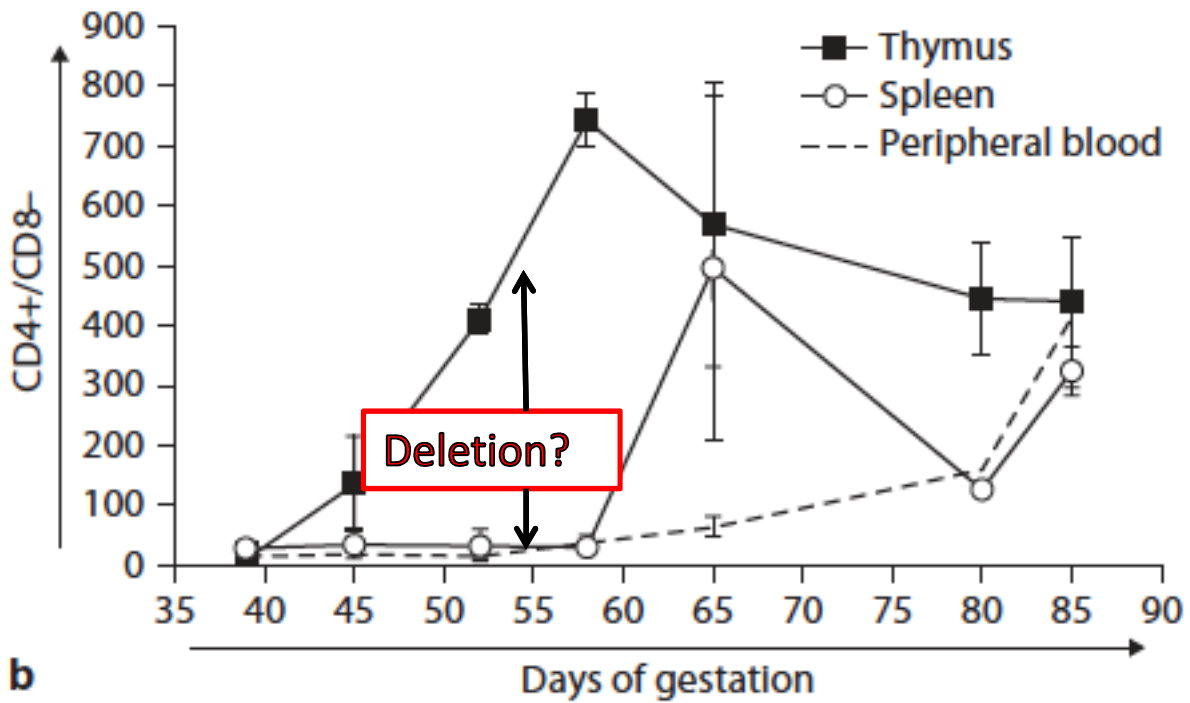
Sheep beyond day 50

Mouse day 14-16

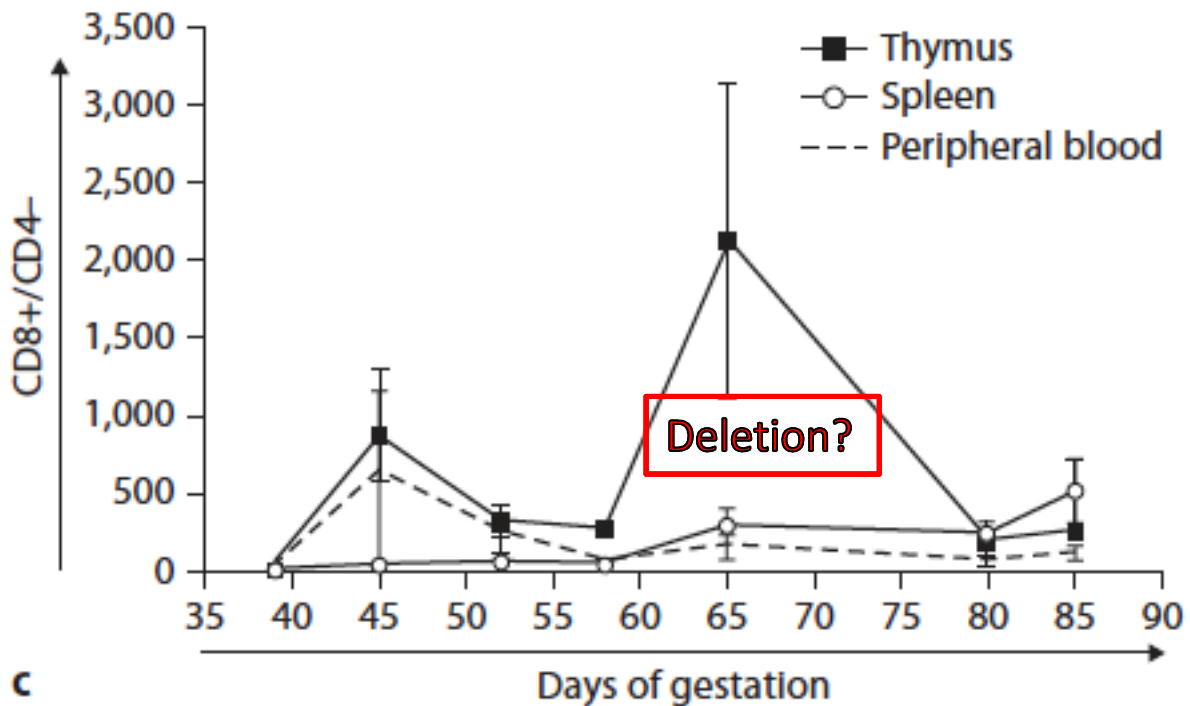
Immunology 1987; 62: 97–105.

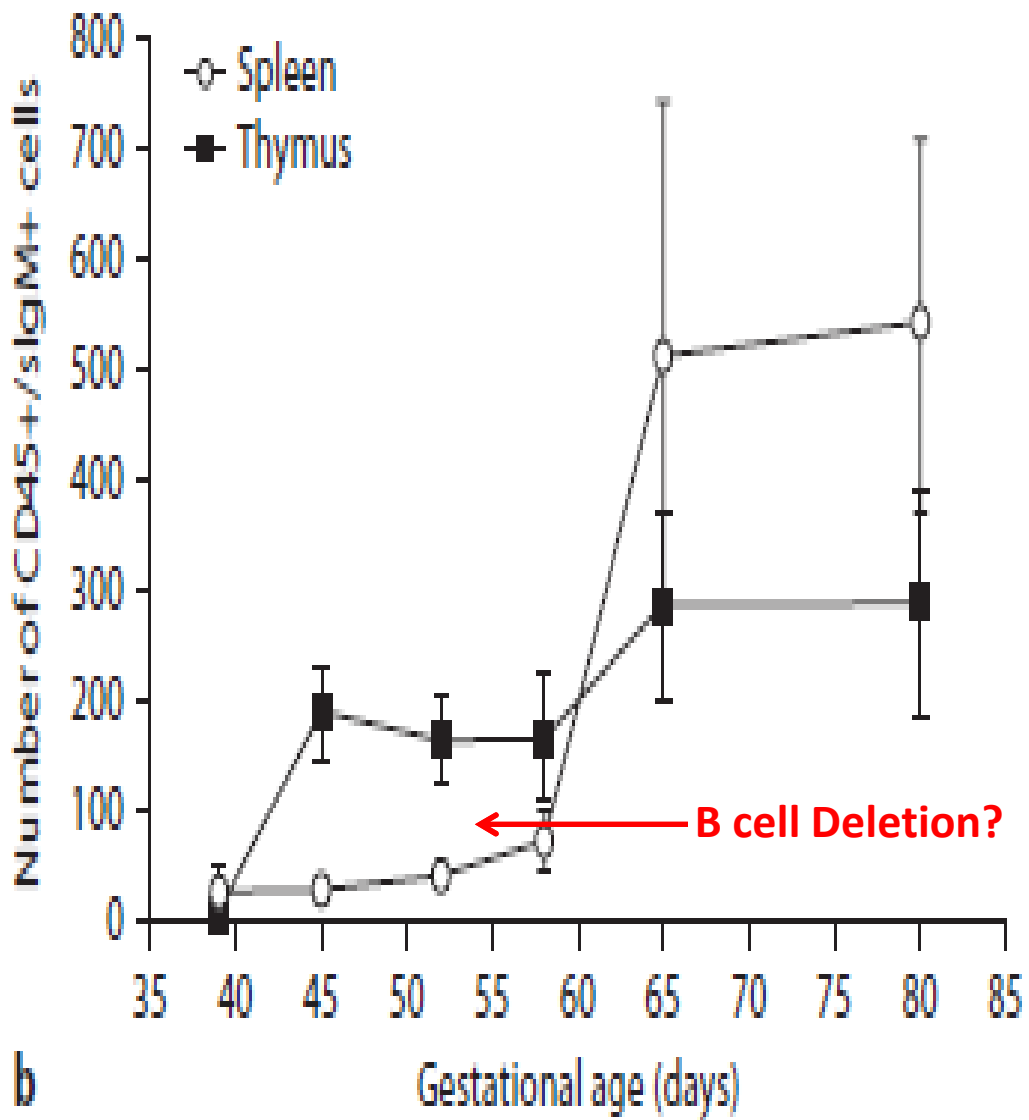
Eur J Immunol 2000; 30:1948-1956 [PMID: 10940884]

CD4+/CD8- Cells

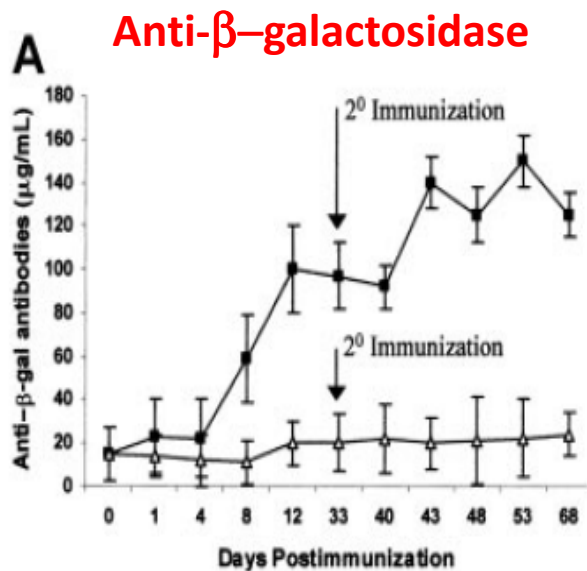


CD4-/CD8+ Cells



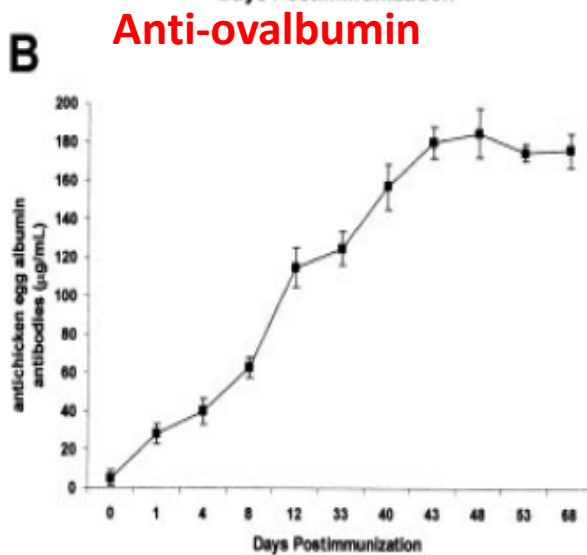


Induction of stable humoral tolerance to β -galactosidase following gene transplantation in utero



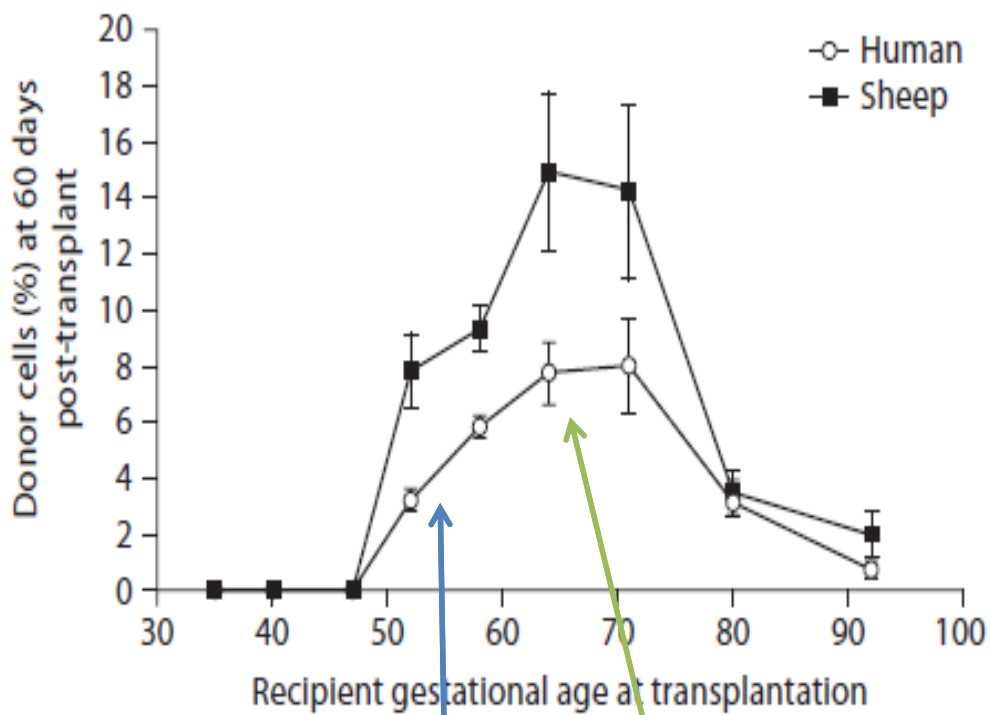
Control sheep

Experimental sheep



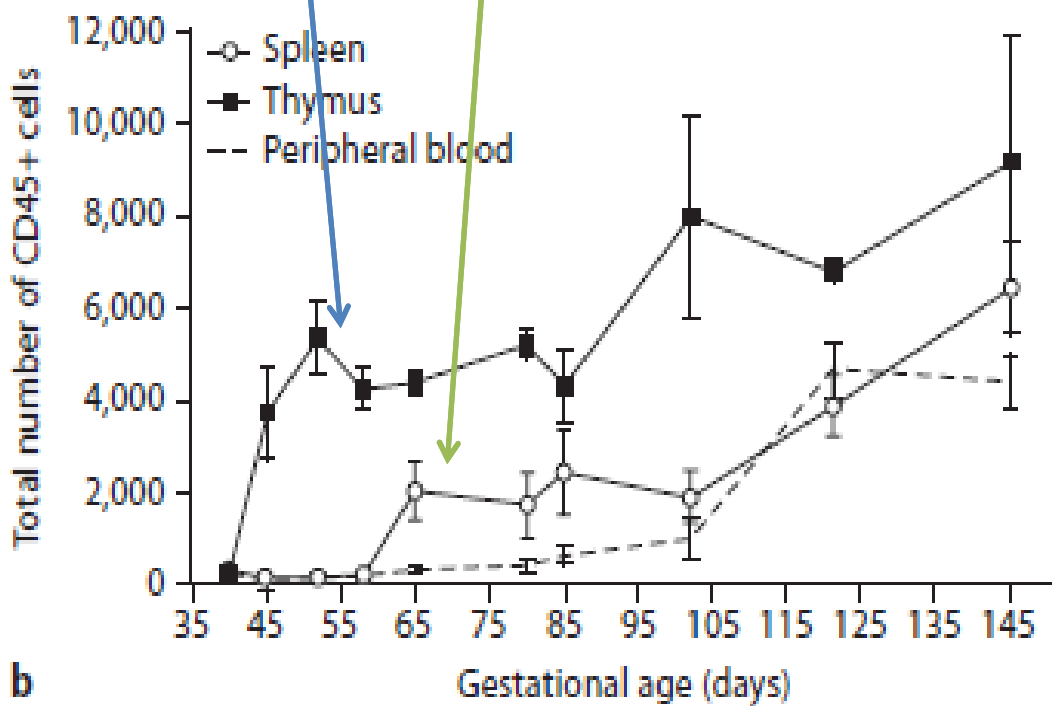
Response to ovalbumin both control and experimental sheep

Anti-ovalbumin



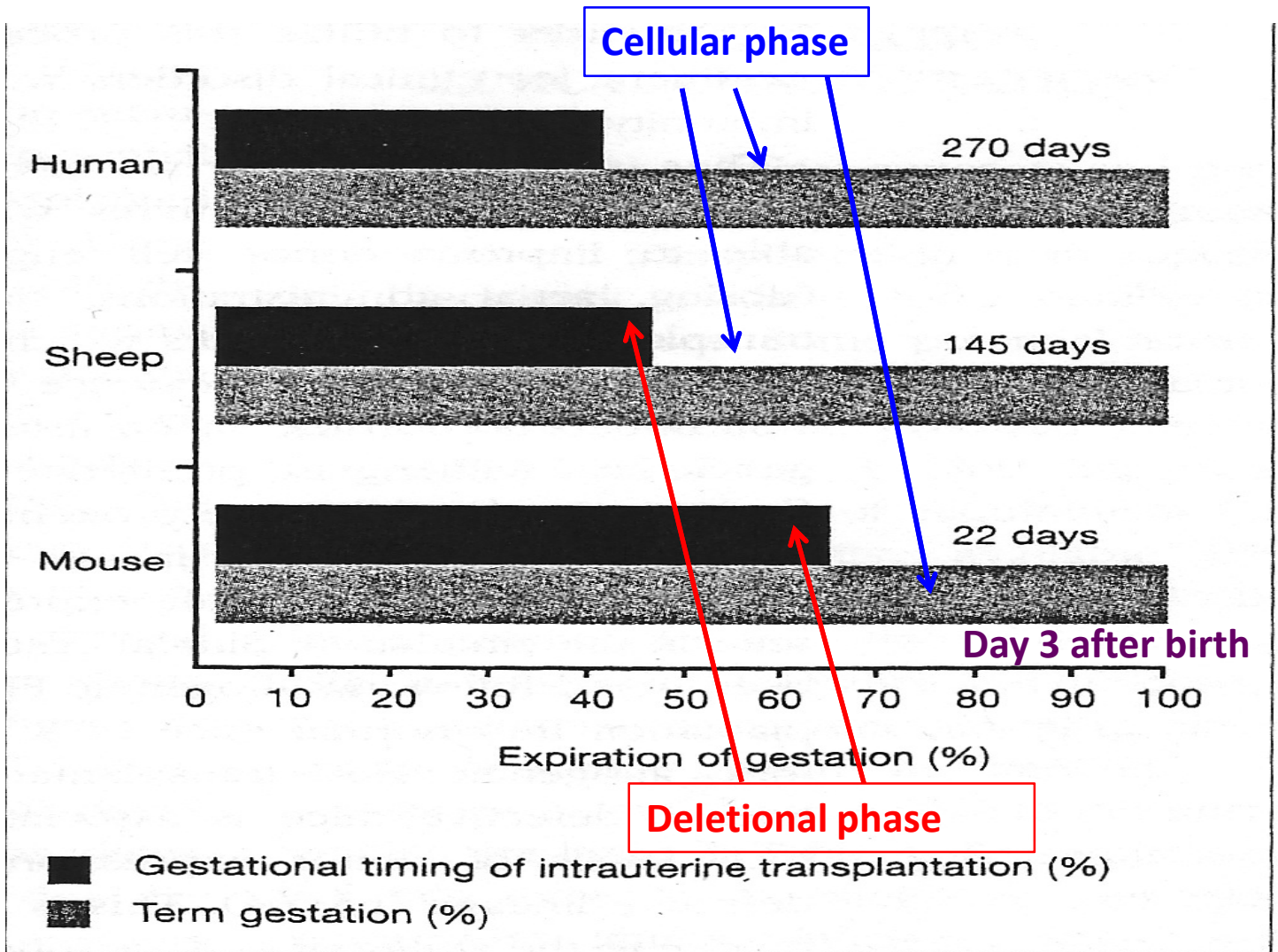
Deletional phase

Cellular phase



b

Fetal tolerance: Deletion phase followed by cellular phase



Fetal transplantation tolerance/self-tolerance requires both the acquisition of deletional and cellular tolerance

- 3 days post thymectomy → autoimmune disease in genetically susceptible mice (*Immunogenetics* 14: 15-27 (1981))
- Thymectomy up to day 3 in mice → Loss of T regulatory cells
Development of autoimmune disease
J Exp Med 161: 72-87 (1985)
- Phenotypic identification of CD4/ CD25 cells in sheep during tolerance window
Experimental Hematology 33 (7) Supplement 1 #351, 2005
- In Utero Bone Marrow Transplantation Induces Donor-Specific Tolerance by a Combination of Clonal Deletion and Clonal Anergy (In mice following allogeneic tx)
J Ped Surgery 34: 726-30 (1999)
- In humans preponderance of T regulatory cell phenotype in third trimester
J of Immunology 152: 3098 (1994)
Science 322: 1562-65 (2008)

Ohki H, Martin C, Corbel C, Coltey M, LeDouarin NM:

Tolerance induced by thymic epithelial grafts in birds.

Science 1987; 237:
1032–1035.

The developing (i.e. fetal) thymic epithelium is not static

Thymus medulla consisting of epithelial islets each derived from a single progenitor

- Constructed chimeric mice by injecting embryonic stem (ES) cells into blastocysts using ES cells and blastocysts differing in their major histocompatibility complex (MHC) type.
- The MHC class-II-positive medullary epithelium in these chimeras is composed of cell clusters, most of which derive from either embryonic stem cell or blastocyst but not mixed origin.
- Thus, the medulla comprises individual epithelial 'islets' each arising from a single progenitor.
- Islet formation can be recapitulated after implantation of "reaggregated fetal thymic organs" into mice, which shows that medullary 'stem' cells retain their potential until at least day 16.5 in fetal development.
- Thus, medulla ± cortex compartmentalization is established by formation of medullary islets from single progenitors.

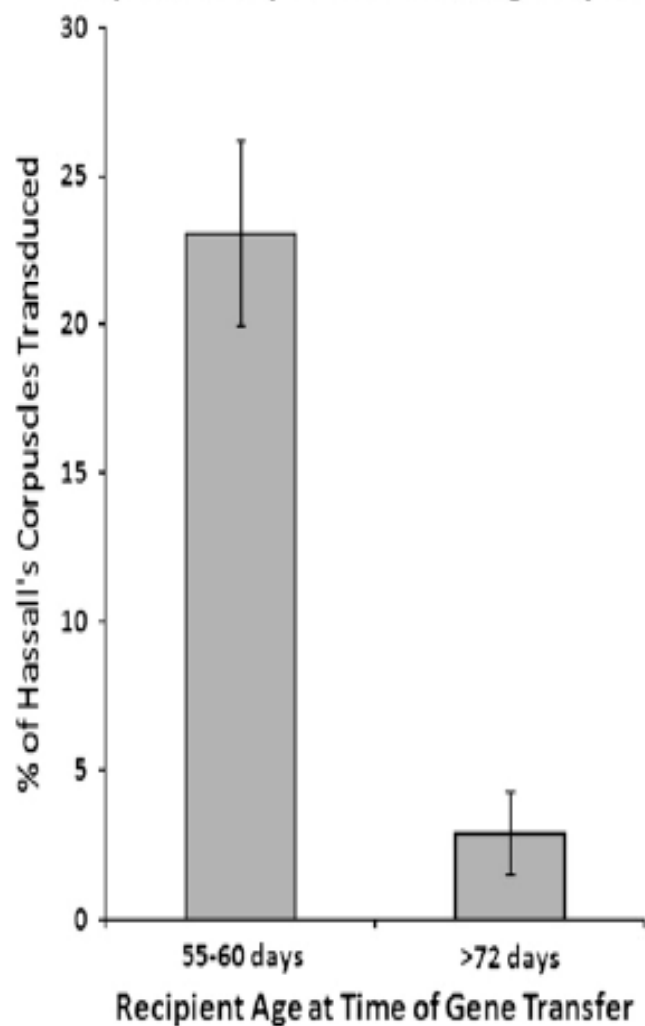
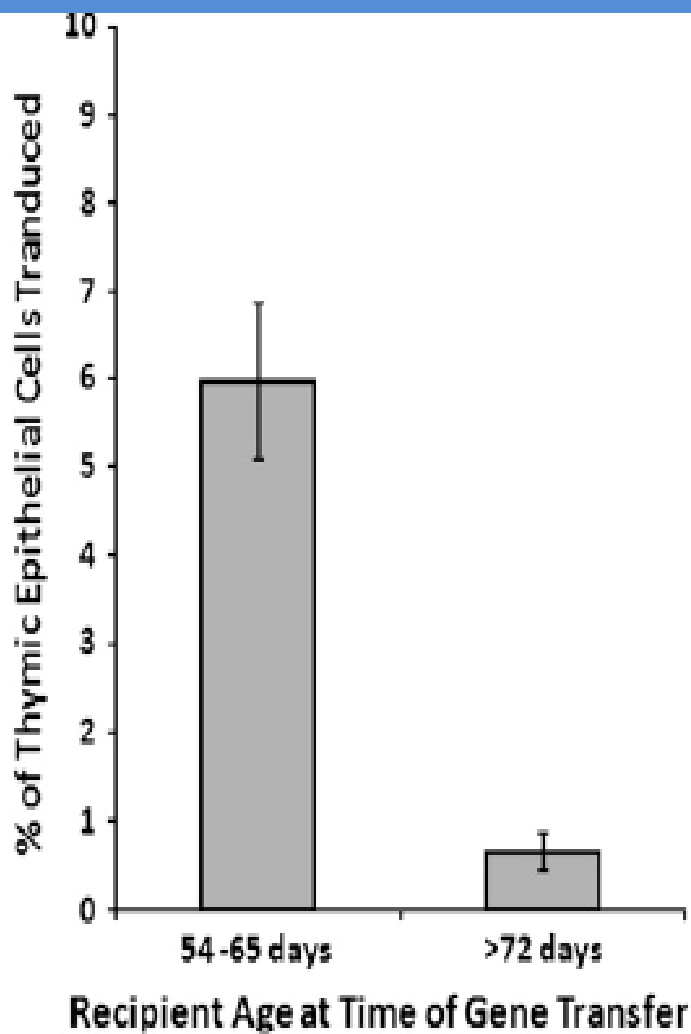
ROLE OF DENDRITIC CELLS!

Nature 414: 763-768 (2001)

Retroviral vector transplantation during the tolerance window is associated with enhanced gene expression in thymic epithelial cells

or

Expression of gene product by thymic epithelial cells is gestational age dependent



What tolerizes the lymphoid compartment?

1. Role of dendritic cells and APCs
2. Thymic epithelial cells (TEC)

Our phenotypic study points to the critical time!
When immune competent cells undergo CD45
differentiation in the thymus after demarcation.

A short note on Natural Killer Cells

Liver biopsy shown earlier

?innate?

Education

Natural Killer Cells: Tolerance to Self and Innate Immunity to Viral Infection and Malignancy

Biol Blood Marrow Transplant 16: S97-S105 (2010)

T cell– and B cell–independent adaptive immunity mediated by natural killer cells

Nature Immunology 7: 507- 516 (2006)

NK-cell education: KIR-S come into play

Blood 115: 1110-1112 (2010)

NK cell development, homeostasis and function: parallels with CD8⁺ T cells

Nature Rev Immun 11: 645-657 (2011)

**NKC development during tolerance window
CD45 isoform differentiation**

IMPRINTING



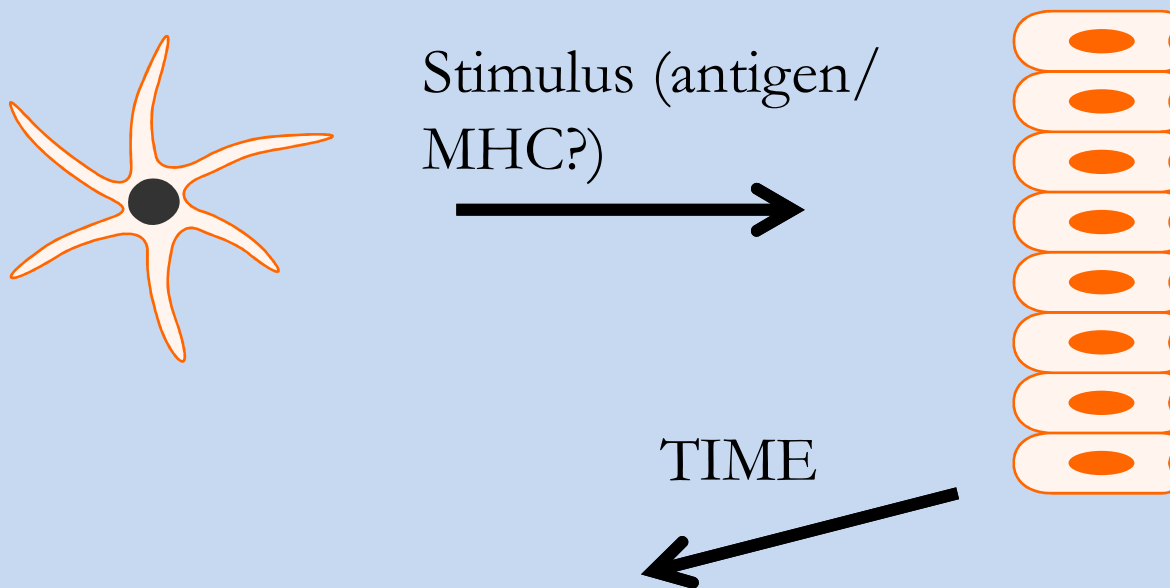
Rapid time-dependent irreversible behavioral learning that occurs during development

“

*Der Kumpan in der Umwelt des Vogels [the companion in the bird's world] Journal fuer Ornithologie. 83: 137-213
Abbreviated translation 1937 in Auk, 54, 245-273*

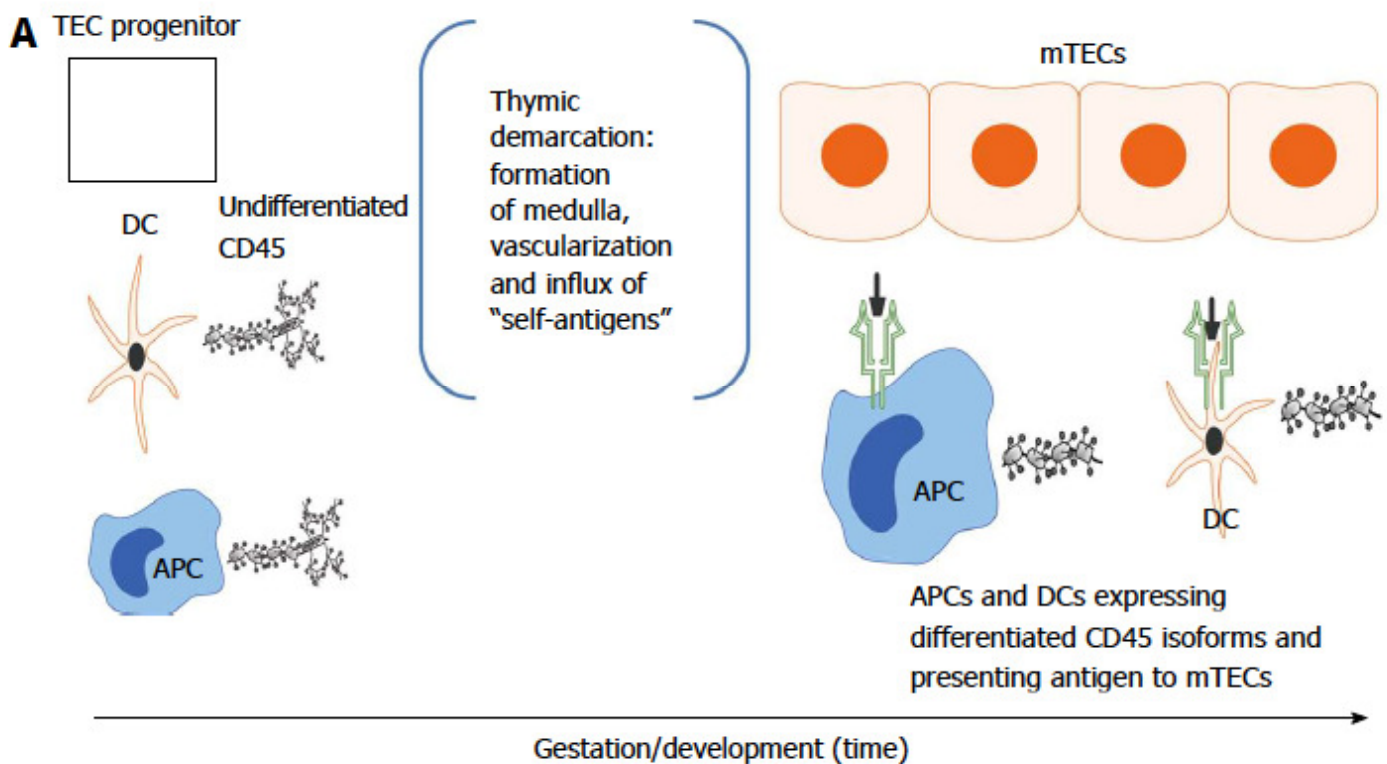
Dr. Pixley's theory of imprinting:

1. During development (i.e. prior to "adulthood")
2. Cell potential response to stimulus is time dependent

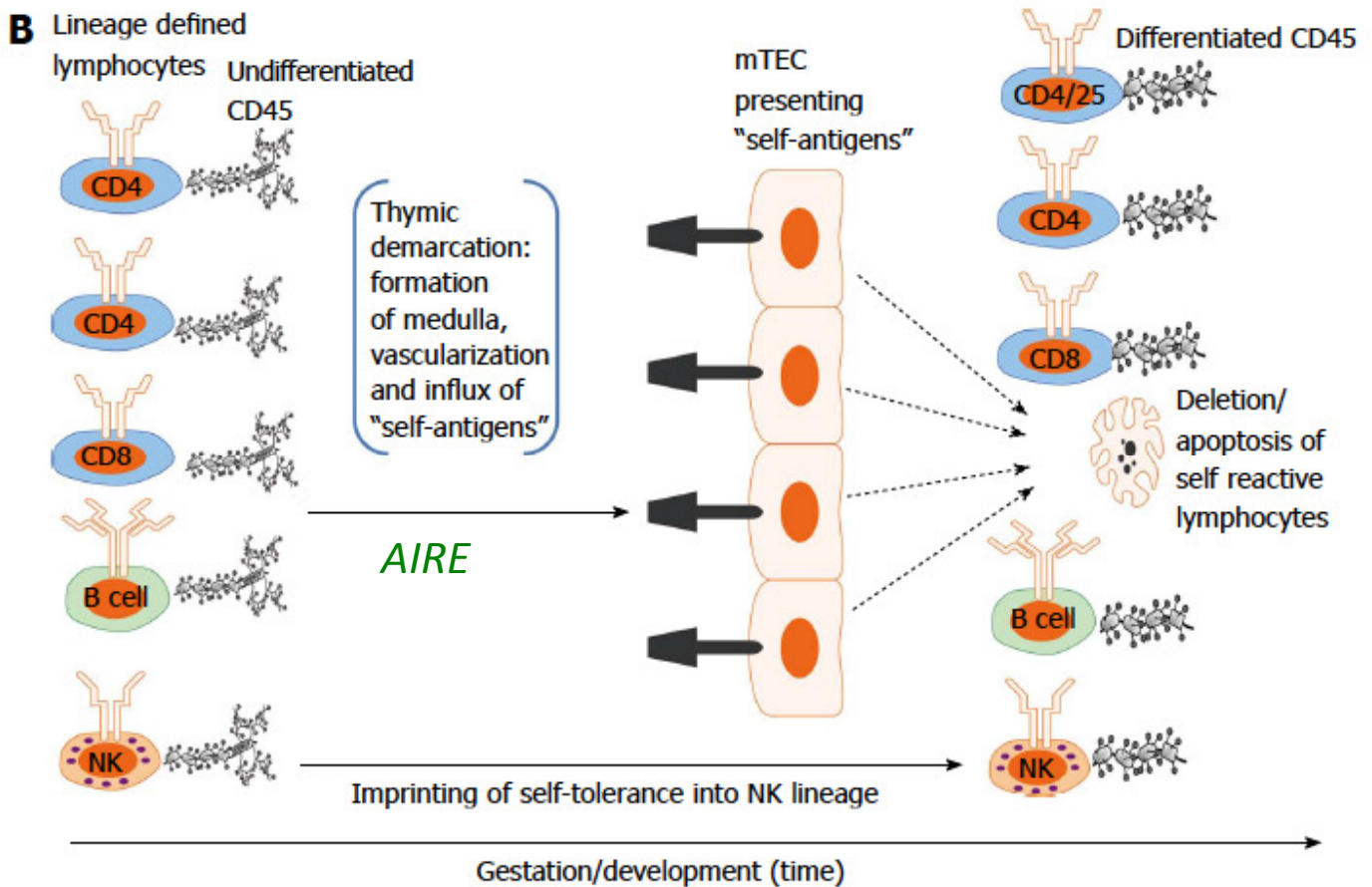


Irreversible Change / self-tolerance
Epigenetic changes to TEC?

Developmental acquisition of self-tolerance: antigen exposure model



Developmental acquisition of self-tolerance: antigen exposure model



Summary

IUT: A powerful investigative method

1. Detailed understanding of mechanisms underlying self-tolerance

2. Applications in therapeutics

Circulating human insulin in sheep!

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	

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