In utero transplantation: (IUT) an experimental window into immune self

Basic and applied implications

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APCs and DCs expressing differentiated CD45 isoforms and presenting antigen to mTECs
“... 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.)

Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins

- 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

*Science, Vol. 102:400-401 (1945)*
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

<table>
<thead>
<tr>
<th>IP injection</th>
<th>IP injection</th>
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<tbody>
<tr>
<td>on day 58-65</td>
<td>on day 14-16</td>
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**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

*J Clin Inv 1992; 89: 1178-88*
Prolonged hematopoietic chimerism in normal mice transplanted in utero with human hematopoietic stem cells

Day 54-65

Day 14-16

Human

Sheep

Mouse

Expiration of gestation (%)

Gestational timing of intrauterine transplantation (%)

Term gestation (%)

Reviews in Clinical and Experimental Hematology 8: 11-32, 1999
Engraftment receptivity is gestational age dependent

ENGRAFTMENT WINDOW: day 52-72

Fetal Diagn Ther 2009;25:102-110
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, Â¬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
Antibody to sheep CD45 recognizes isoforms: MW 225-190 Kilodaltons
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

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.

Fetal Diagn Ther 2009;25:102-110
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


CD4+/CD8- Cells

CD4-/CD8+ Cells

Fetal Diagn Ther 2009;25:102-110
Induction of stable humoral tolerance to β-galactosidase following gene transplantation in utero

Blood 2001 97;(7): 3417-23
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 $\rightarrow$ autoimmune disease in genetically susceptible mice (Immunogenetics 14: 15-27 (1981))
- Thymectomy up to day 3 in mice $\rightarrow$ Loss of T regulatory cells
  Development of autoimmune disease
- 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)
- In humans preponderance of T regulatory cell phenotype in third trimester
  J of Immunology 152: 3098 (1994)
Ohki H, Martin C, Corbel C, Coltey M, LeDouarin NM:

Tolerance induced by thymic epithelial grafts in birds.


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

Exp Hematol 2008 36:816-22
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

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


NK cell development during tolerance window

CD45 isoform differentiation
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

Stimulus (antigen/MHC?)

Irreversible Change / self-tolerance
Epigenetic changes to TEC?

TIME
Developmental acquisition of self-tolerance: antigen exposure model

World J Stem Cells 2013 April 26; 5(2): 43-52
doi:10.4252/wjsc.v5.i2.43
Developmental acquisition of self-tolerance: antigen exposure model

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

IUT: A powerful investigative method

1. Detailed understanding of mechanisms underlying self-tolerance

2. Applications in therapeutics
Circulating human insulin in sheep!

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<th>When sampled (months post-transplant)</th>
<th>Human C-peptide (ng/mL)</th>
<th>CV%</th>
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