The uterine mucosal immune system

Ashley Moffett
University of Cambridge

Decidual Leukocytes

- 70% CD56^{bright} NK cells
- 20% CD14^{+} Macrophages
- 1% Dendritic Cells

(both HLA-DR+ myelomonocytic cells)

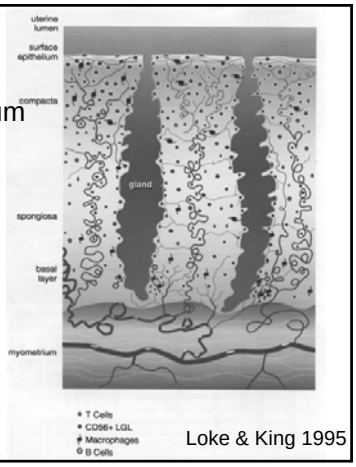
- 10% T cells
- Rare B cells

Leukocytes in secretory endometrium

NK cells aggregate around spiral arteries and glands In functional layer

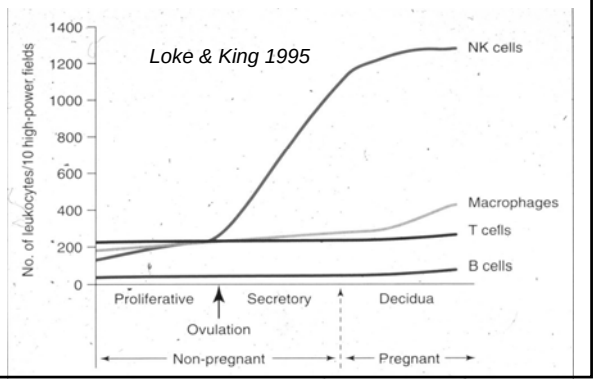
T and B cells present in basal aggregates

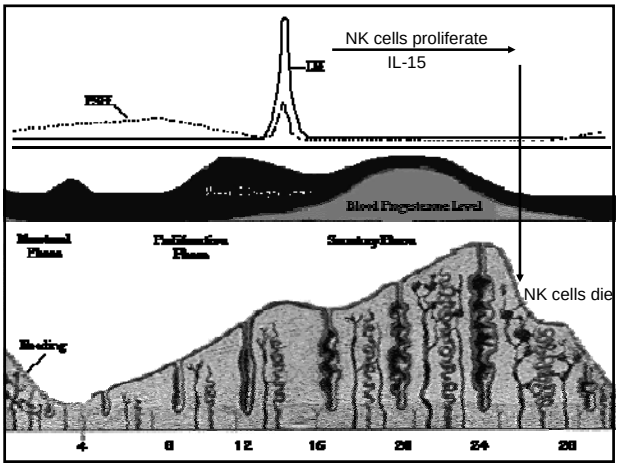
T cells and macrophages scattered throughout stroma

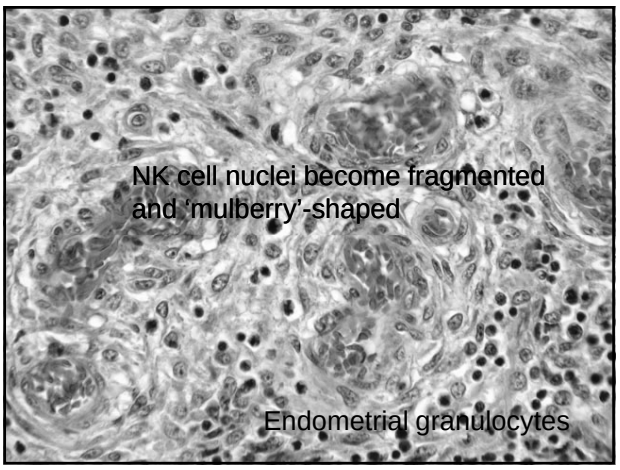


Loke & King 1995

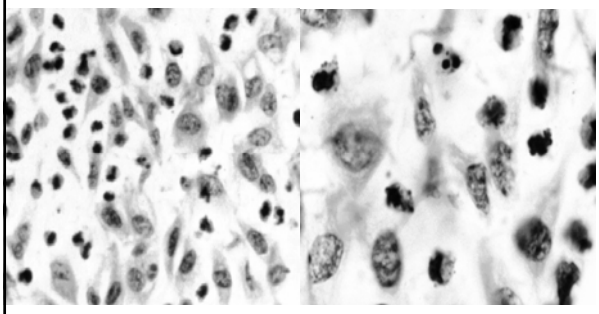
NK cells in endometrium and decidua







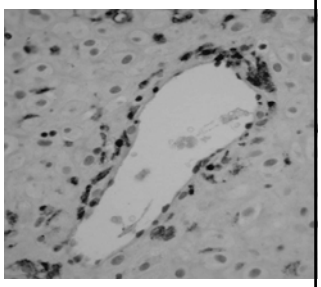
Phloxine tartrazine staining of endometrium after P withdrawal



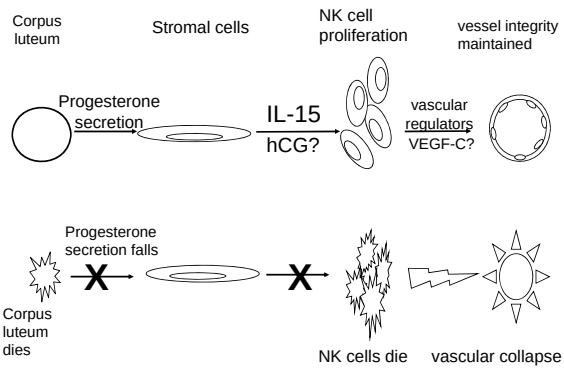
•Caspase-independent cell death.
•Mitotic catastrophe?

Do uNK cells regulate vasculature?

- **Make many angiogenic growth factors - VEGF, PlGF, NKG5**
- **Perivascular location**
- **Increased in women bleeding on HRT**



Do uNK cells trigger mucosal breakdown?



The Yin and Yang of the Endometrium

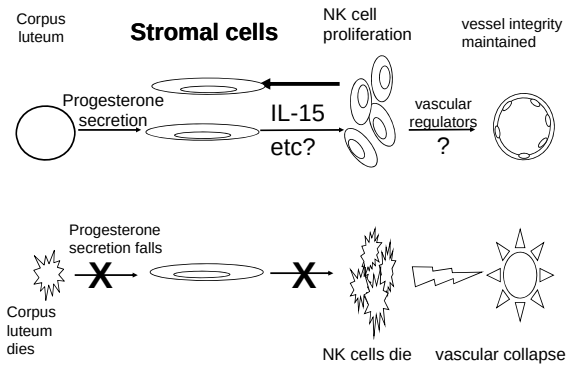
Decidualisation

- Endometrium continues to decidualise after ~day 24 of menstrual cycle
- NK cells always present

Disintegration

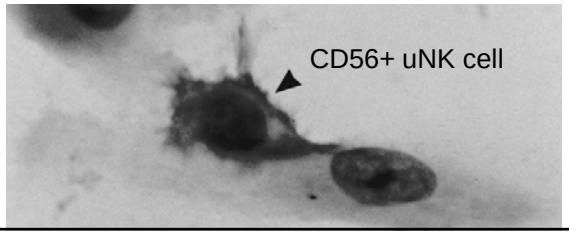
- Endometrium breaks down at menstruation
- NK cells die on day 26-27 preceding any other change signifying menstruation

Do uNK cells affect stromal cells?



Do NK cells aid decidualisation process?

- NK cells always integral part of decidua
- Not present in species with epitheliochorial placentation
- Not present in Fallopian tube in ectopic pregnancy
- In mice with no uterine NK cells, decidua is deficient



Paracrine effects of uterine leucocytes on gene expression of human uterine stromal fibroblasts

Ariane Germeyer, Andrew Sharkey, Mirari Prasadajudio, Robert Sherwin, Ashley Moffett, Karen Bieback, Susanne Clausmeyer, Leanne Masters, Roxana Maria Popovici, Alexandra Petra Hess, Thomas Strowitzki and Michael von Wolff

Molecular Human Reproduction 2009 15(1):39

No effect of uterine NK cells on conventional markers of decidualisation

BUT

NK supernatants cause increase in transcription of many genes

including

IL-7R, IL-8, IRF-1, IL-15 and IL-15R

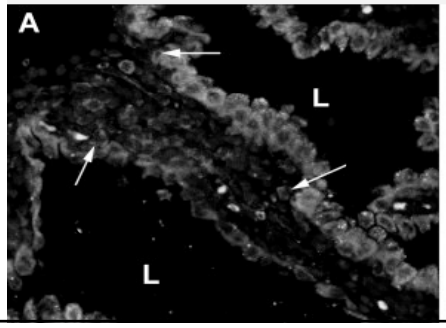
NK cells may affect trophoblast migration

Express factors important in creating their own niche

Reprod Biol Endocrinol. 2004; 2: 58.

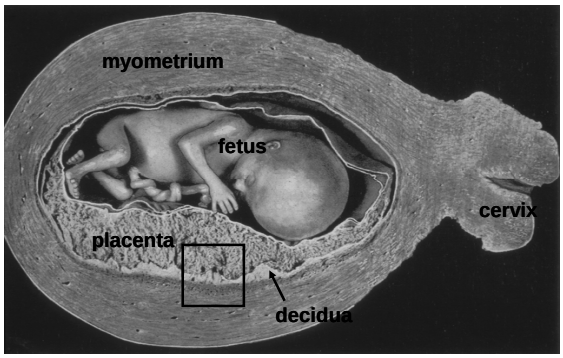
Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: A morphological and immunohistochemical study

Joanne Hempstock,Tereza Cindrova-Davies,Eric Jauniaux,Graham J Burton



Functions of uterine NK cells

- May have physiological role in regulating mucosal growth and breakdown by influencing function of vasculature, stromal cells and glands.
- Mediate allorecognition of trophoblast cells and control placentation



Leukocytes in secretory endometrium

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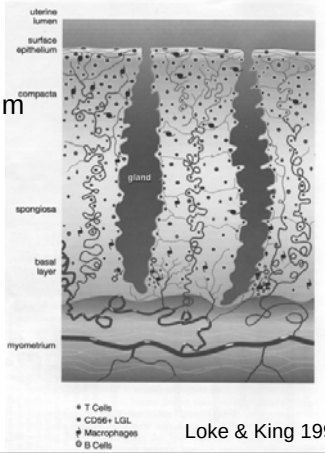
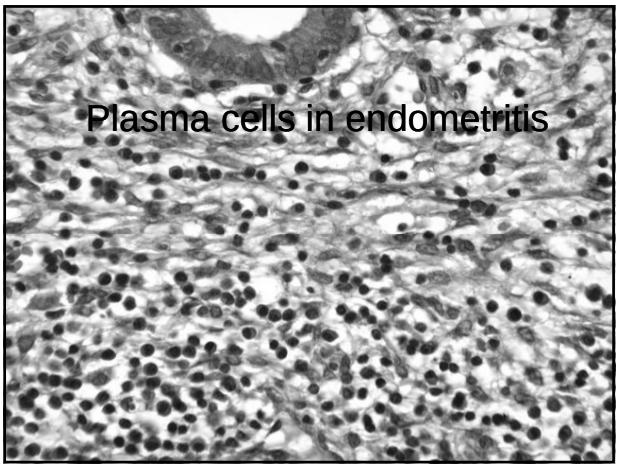
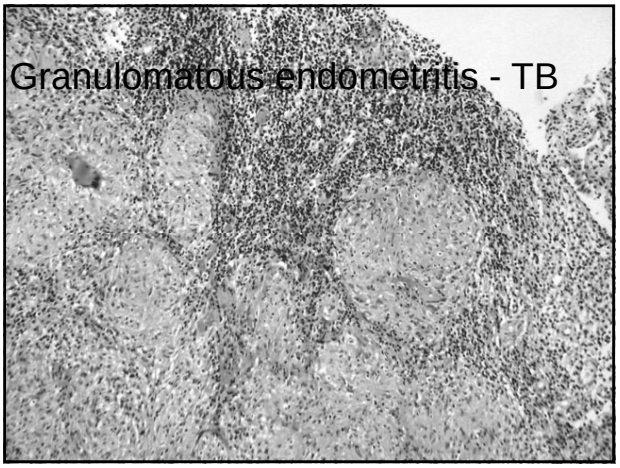


Loke & King 1995





1953

SOME IMMUNOLOGICAL AND ENDOCRINOLOGICAL PROBLEMS RAISED BY THE EVOLUTION OF VIVIPARITY IN VERTEBRATES

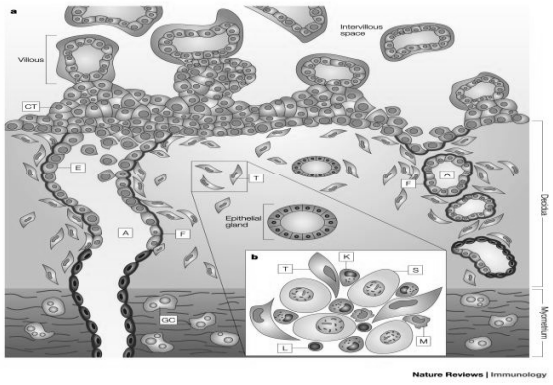
By P. B. MEDAWAR

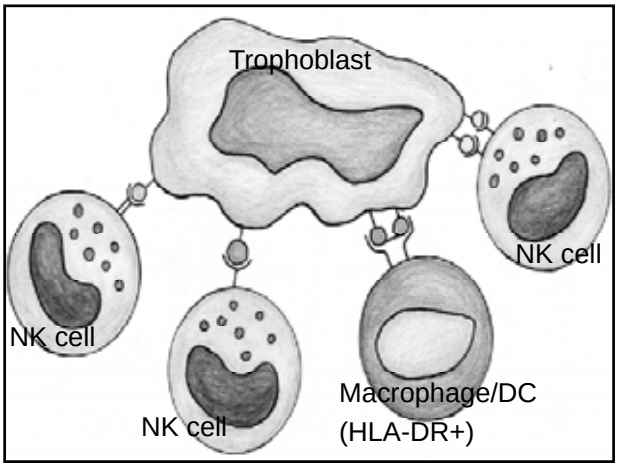
Department of Zoology, University College, London

Recipient helper T cell	Recipient cytotoxic T cell
CD4 T cell	CD8 T cell
Allograft	Allograft
Donor MHC antigens Class II HLA-DR	**Donor MHC antigens Class I HLA-A. HLA-B**

The anatomy of placental implantation

Moffett 2002 NRI





HLA class I molecules

Classical HLA A, B, C

Non-classical HLA E, F, G

Problems:

•Trophoblast cells in humans are difficult to isolate

•HLA-I molecules are very similar so PCR primers, mAbs etc will cross-react.

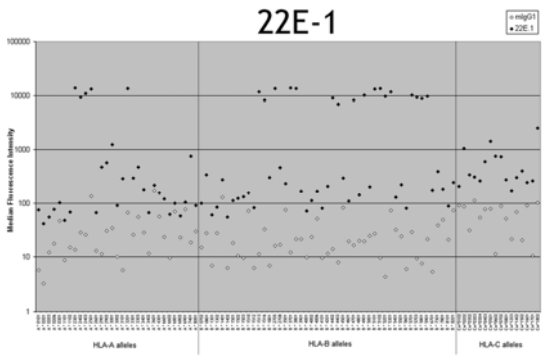
NB. **No** HLA-DR Class II molecules ever found on any trophoblast cell

IMMUNOLOGY ORIGINAL ARTICLE

Human leucocyte antigen (HLA) expression by normal trophoblast cells and placental cell lines, determined using a novel method to characterize allotype specificities of anti-HLA antibodies

Richard Apps,[1] Shawn P. Murphy,[2] Raymond Fernando,[3] Lucy Gardner,[1] Tashmeeta Ahad[1] and Ashley Moffett[1]

Immunology 2009 In press



Characterisation of one of the mAbs as an example

Trophoblast HLA

HLA class I molecules found on most nucleated cells

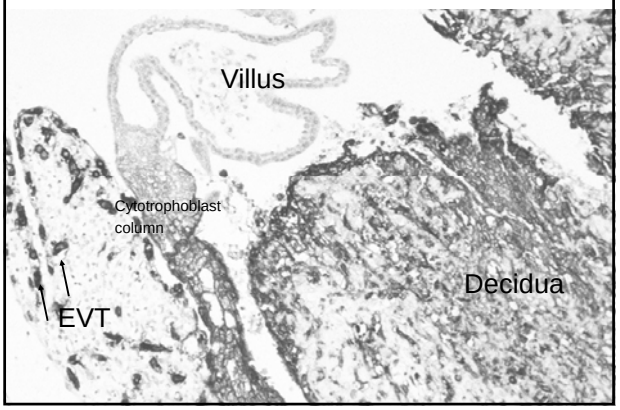
A B C E F

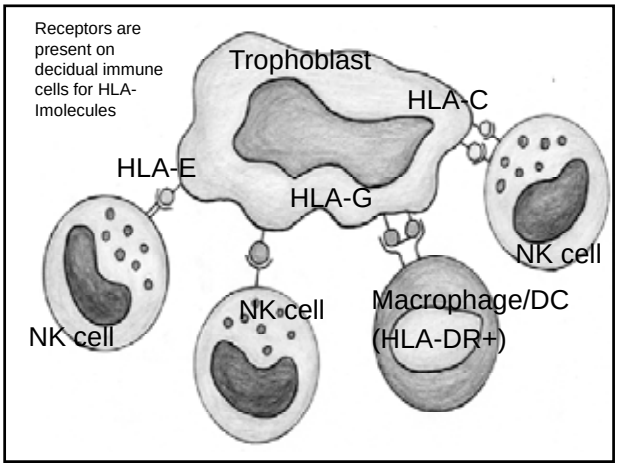
- Extravillous trophoblast HLA class I molecules

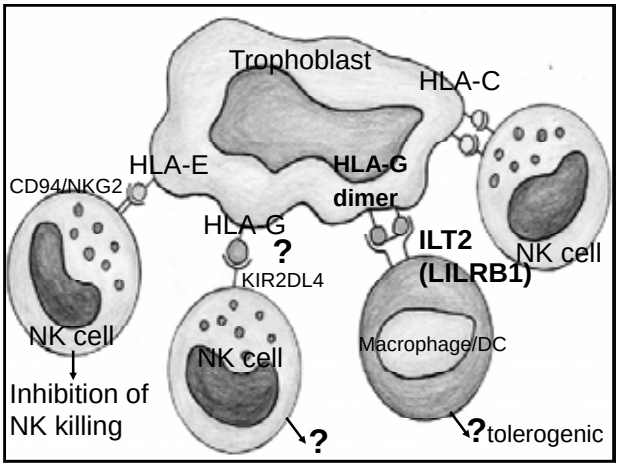
C E **G**

- Villous trophoblast HLA-negative

HLA-G expression by extravillous trophoblast





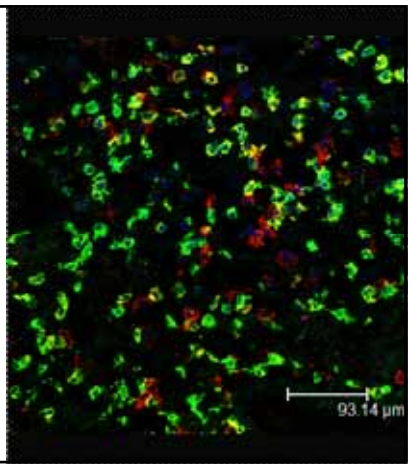


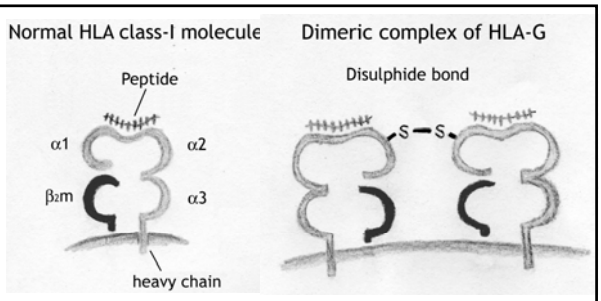
Decidua basalis

CD56+ NK cells green

HLA-DR+ cells LILRB1 red

CD56+, LILRB+ yellow



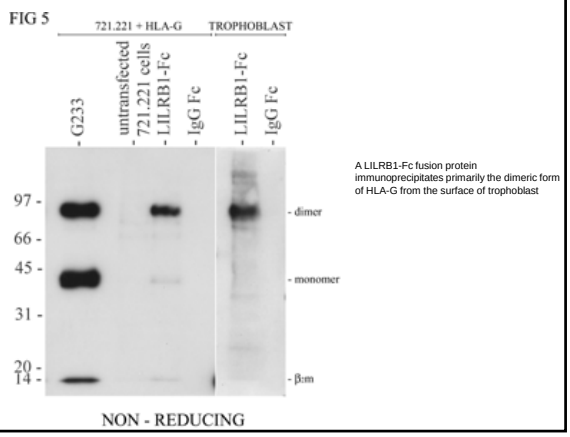


A homodimeric complex of HLA-G on normal trophoblast modulates antigen-presenting cells *via* LILRB1

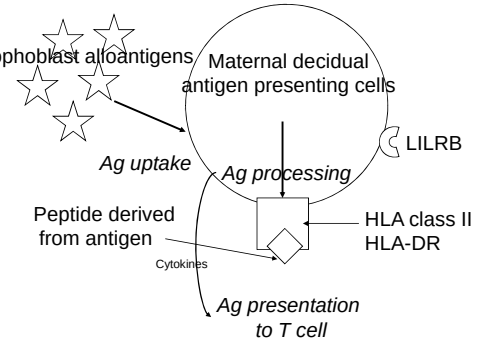
Richard Apps, Lucy Gardner, Andrew M. Sharkey, Nick Holmes and Ashley Moffett

Eur. J. Immunol 2007

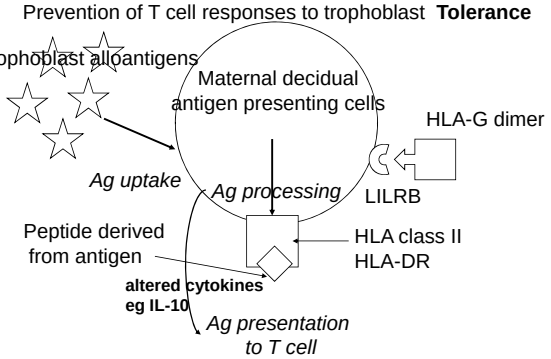
LILRB1 binds preferentially the dimeric form of HLA-G at the surface of trophoblast

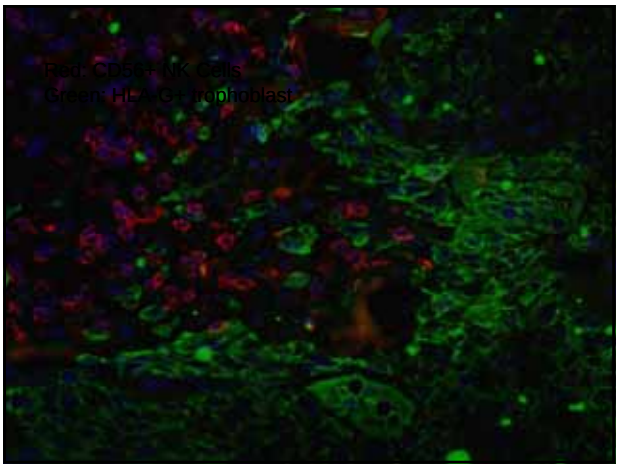


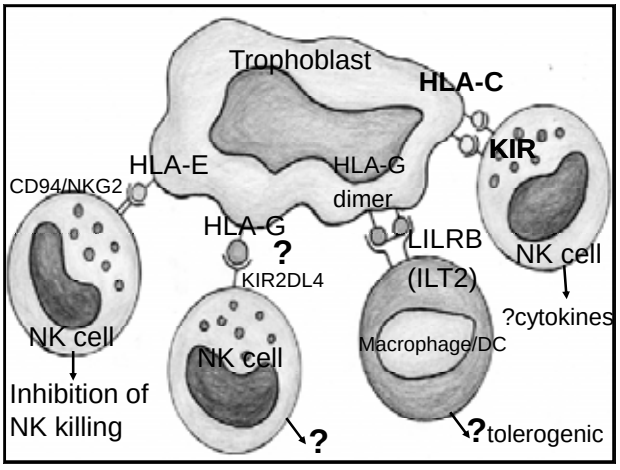
Generation of T cell responses to trophoblast

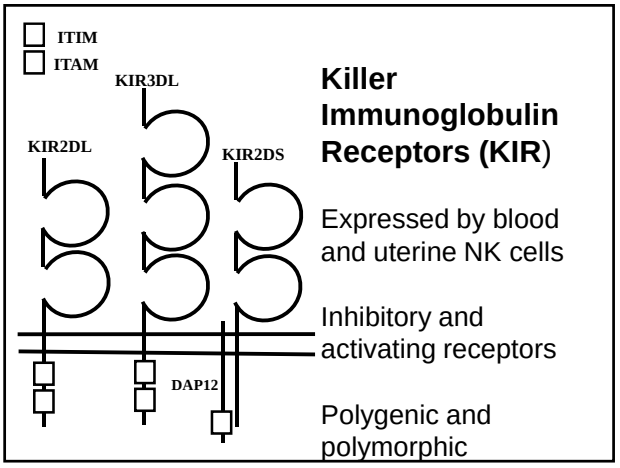


Prevention of T cell responses to trophoblast **Tolerance**

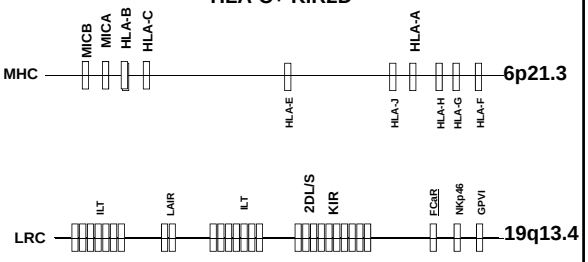



Red: CD56+ NK Cells
Green: HLA-G+ trophoblast

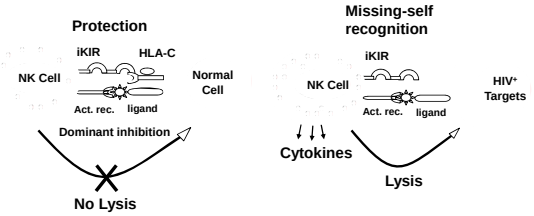

Trophoblast
HLA-C
KIR
HLA-E
HLA-G
dimer
CD94/NKG2
HLA-G
?
KIR2DL4
LILRB
(ILT2)
NK cell
?cytokines
NK cell
Macrophage/DC
NK cell
Inhibition of
NK killing
?
?tolerogenic


ITIM
ITAM
KIR3DL
KIR2DL
KIR2DS
DAP12
Killer Immunoglobulin Receptors (KIR)
Expressed by blood and uterine NK cells
Inhibitory and activating receptors
Polygenic and polymorphic

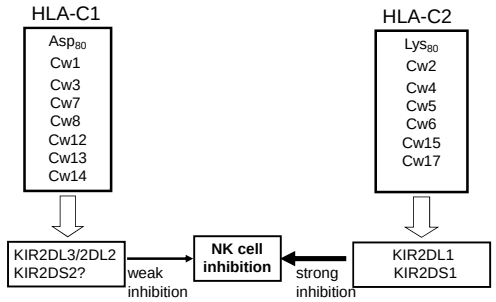
HLA class I and KIR: functionally related gene clusters exhibiting extreme polymorphism
HLA-C+ KIR2D



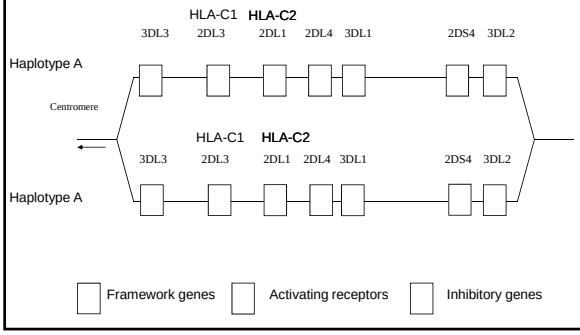
KIR regulate NK cell activity under normal and aberrant conditions



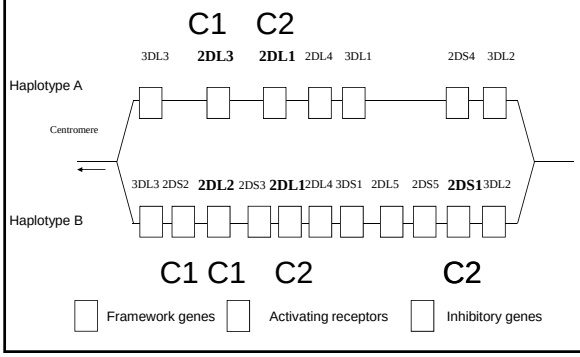
HLA-C Dimorphism



The AA KIR genotype



Two representative KIR haplotypes of A and B type



Killer Ig-Like Receptor Expression in Uterine NK Cells Is Biased toward Recognition of HLA-C and Alters with Gestational Age[1]

Andrew M. Sharkey,* Lucy Gardner,* Susan Hiby,* Lydia Farrell,* Richard Apps,* Leanne Masters,* Jodie Goodridge,† Louise Lathbury,‡ C. Andrew Stewart,[2‡] Sanjay Verma,* and Ashley Moffett[3*]

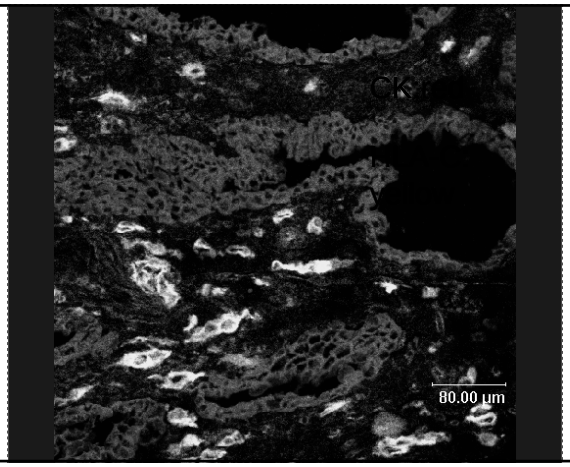
J. Immunology 2008

Conformation of human leucocyte antigen-C molecules at the surface of human trophoblast cells

Richard Apps, Lucy Gardner, Sue E. Hiby, Andrew M. Sharkey and Ashley Moffett

Department of Pathology, Tennis Court Road, Cambridge, UK

Immunology 2008



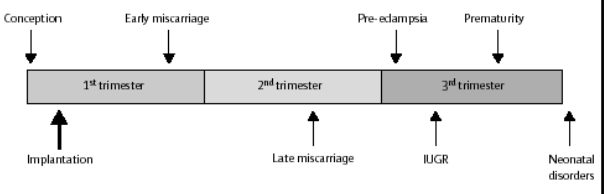
KIR and HLA-C are both polymorphic

In each pregnancy:

- KIR repertoire will be specific for each mother
- Fetal HLA-C (group C1 or C2) will be specific for paternal and maternal HLA-C contribution

Are certain combinations of maternal KIR and fetal HLA-C genotypes associated with failure of placentation?

Spectrum of reproductive failure during pregnancy



Do certain combinations of KIR and HLA-C increase risk of pre-eclampsia?

Mother's KIR

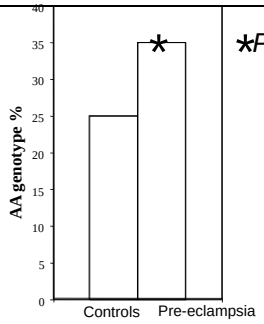
A
A

A
B

B
B

Frequency of AA Genotype in normal and pre-eclamptic mothers

Hiby et al 2004
J.Exp Med



**P*=0.035

Do certain combinations of KIR and HLA-C increase risk of pre-eclampsia?

Mother's KIR

Baby HLA-C

C1 C1 | C1 C2 | C2 C2

A
A

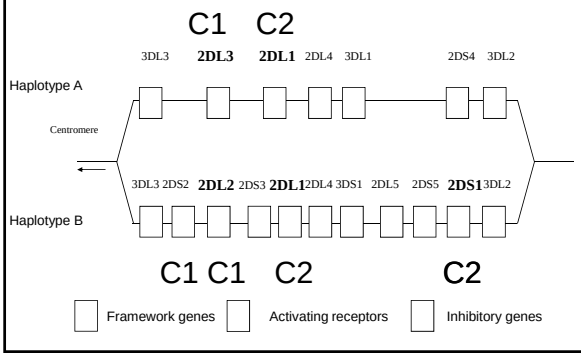
A
B

B
B

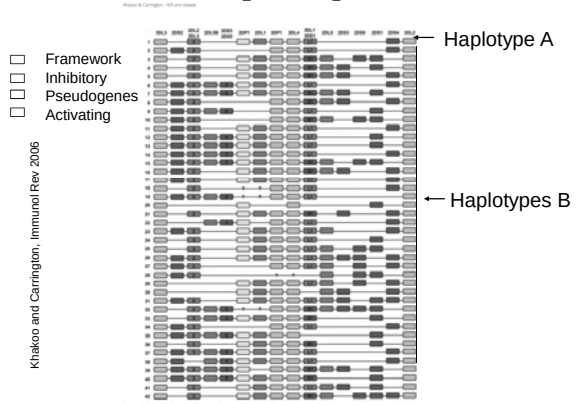
Certain combinations of KIR and HLA-C increase risk of pre-eclampsia

Mother's	Baby HLA-C: C1 C1	C1 C2	C2 C2
A A	good	bad	bad
A B	good	good	good
B B	good	good	good

Two representative KIR haplotypes of A and B type

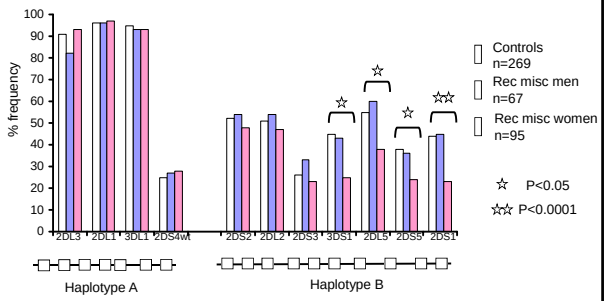


NK cell receptor repertoire



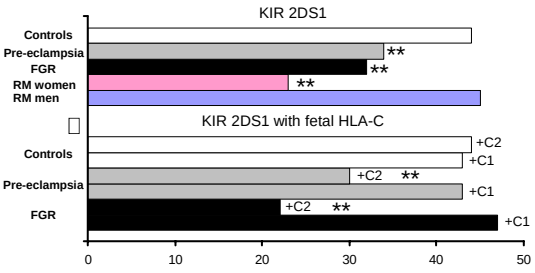
Recurrent Miscarriage

Telomeric genes of the KIR B haplotype are lacking in recurrent miscarriage women but not their male partners



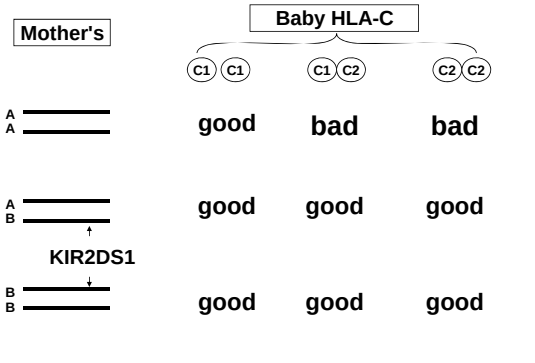
(Hiby et al 2008)

Maternal KIR2DS1 frequencies are significantly decreased in reproductive failure

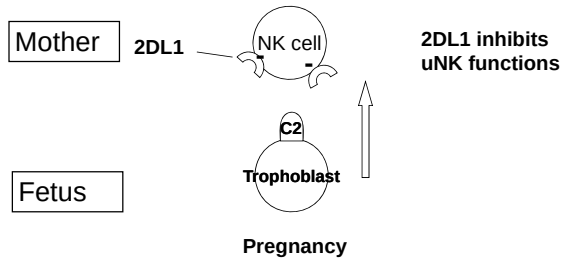


$^{**}p \leq 0.01$ (comparing frequencies of affected with controls)

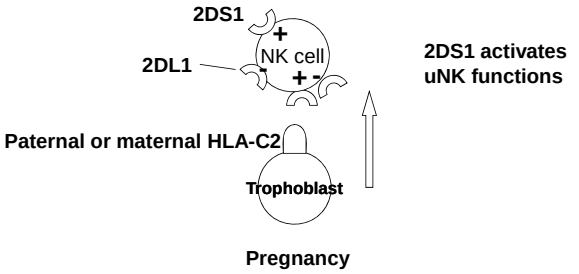
Certain combinations of KIR and HLA-C increase risk of pre-eclampsia

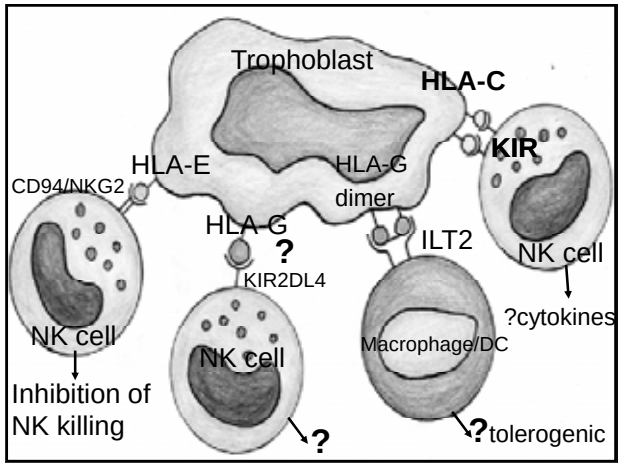


2DL1/C2 is associated with reproductive failure



2DS1 protects against reproductive failure if C2 in baby





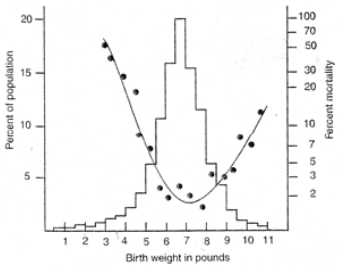
Sue Hiby
Andrew Sharkey
Richard Apps

Lucy Gardner
Viki Male
Leanne Masters
Lydia Farrell

Collaborators:
Mary Carrington
John Trowsdale
Charlie Loke

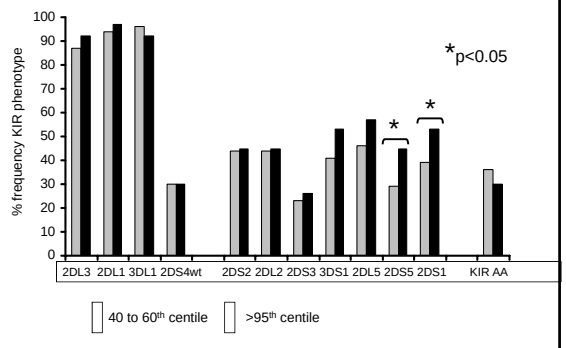
Chris Redman
Jimmy Walker
Linda Morgan
Lesley Regan
Per Magnus

The distributions of birthweight and early mortality rate
(n=13,730)



The data indicate stabilizing selection on birth weight since the optimum birth weight is associated with the lowest mortality (from Cavalli-Sforza, L.L. & Bodmer, W.F. In *The genetics of human populations*. (Freeman Press, San Francisco, 1971).)

B haplotype telomeric KIR increased in frequency in the mothers of the large babies compared to the controls (Norwegian MoBa cohort)



KIR and HLA-C and evolution

KIR only expanded in primates

Increase in activating KIR receptors in humans

Population differences in KIR gene frequencies

MHC-C1 appears in some orang-utangs
MHC-C2 only in great apes (not orang-utangs)