Stanovení HLA znaků asociovaných s chorobami
workshop 2023

16/2/2023

HLA and KIR in reproduction

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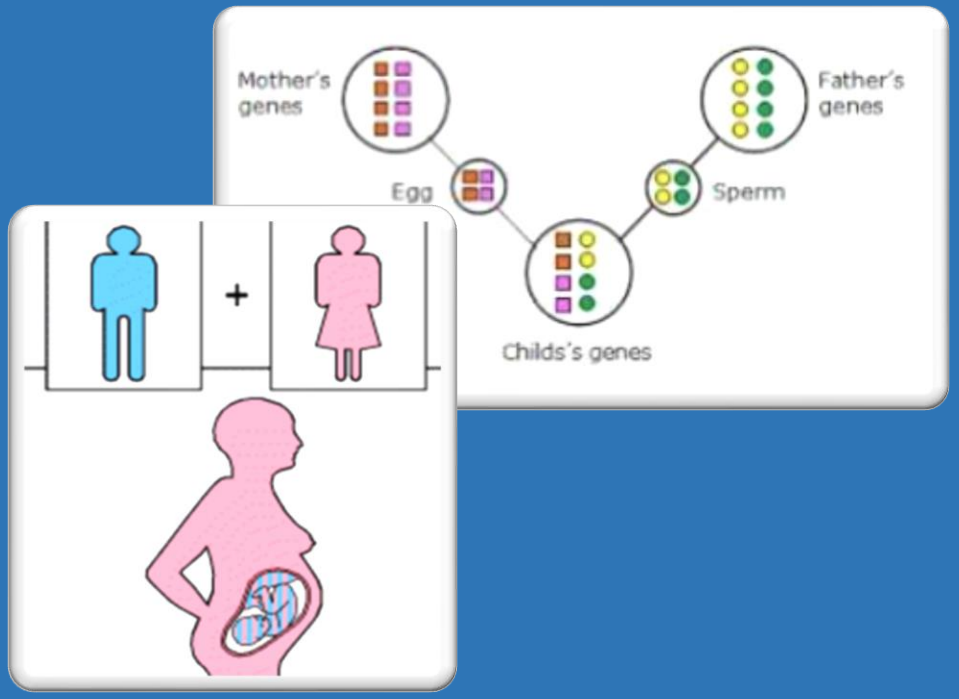
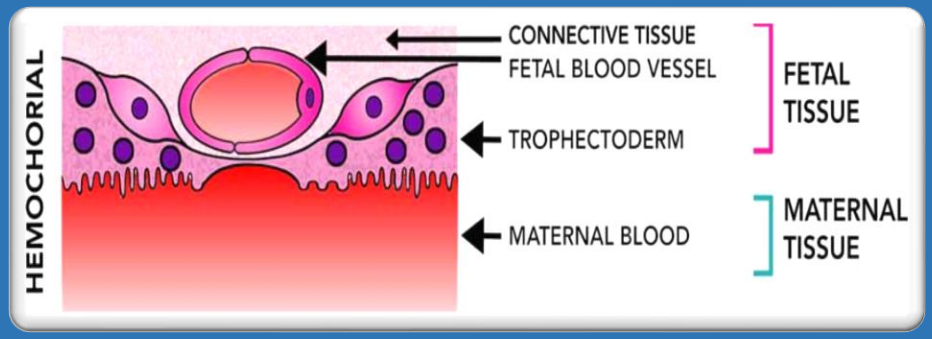
The fetus is a **semiallograft**
half of its antigenic make-up comes from the mother and half from the father



The maternal leukocytes are in continuous contact with the fetal tissues lining the maternal vessels of the deciduas and placenta



There are several sites and times at which the maternal immune system may be challenged with fetal/paternal alloantigens

The maternal immune system does <u>not</u> reject the fetus

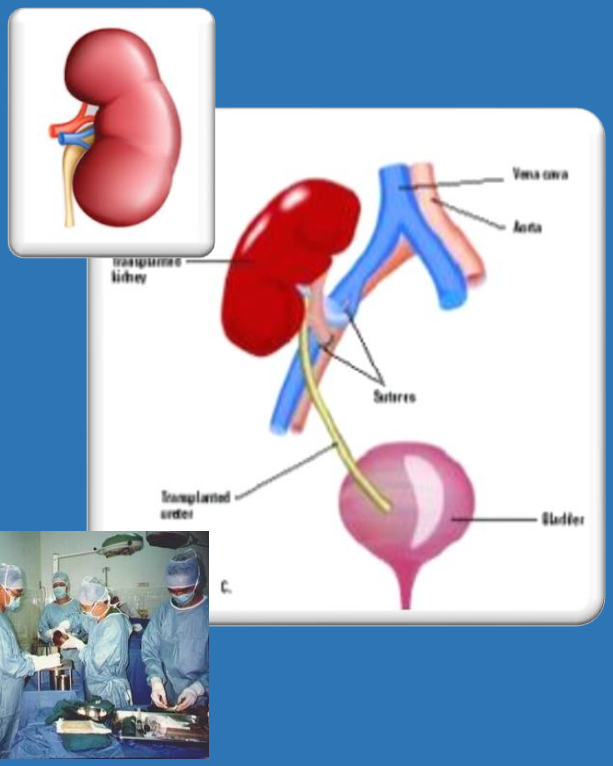
«How does the pregnant mother continue to nourish within herself for many weeks or months a foetus that is antigenically a foreign body?»

(1953)

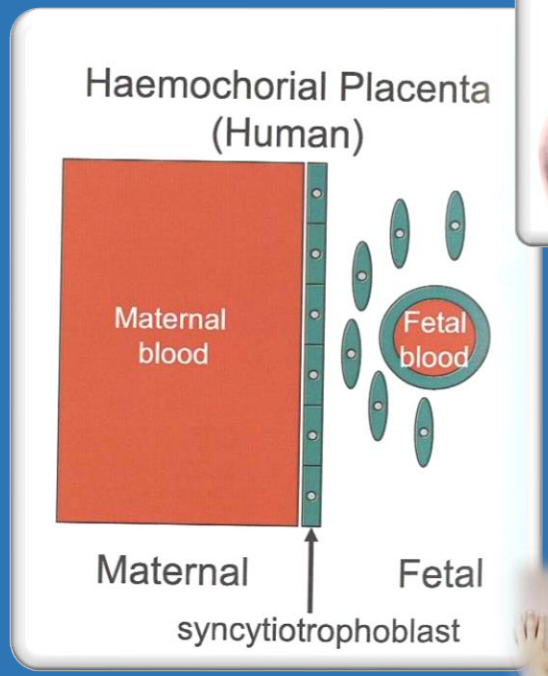
Sir Peter Medawar
and
Rupert Billingham

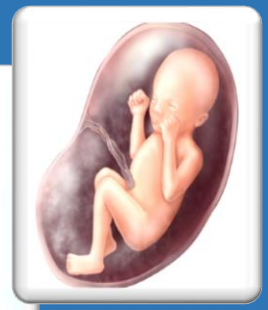
The Immunological Paradox of Pregnancy

The embryo is not a transplant



- unnatural medical/therapeutical manipulation
- transplanted organ or tissues remain invariable regarding structure and genetic characteristics
- transplanted organ is directly exposed to the host/recipient blood circulation
- host/recipient's organism is not naturally prepared to accept the graft





- physiological procedure/function
- embryo/fetus undergoes alterations
- mother and fetus blood do not mix and substances are exchanged through the placenta by diffusion ("hemochorial" placentation)
- maternal organism is prepared (hormone mediated changes) for blastocyst/embryo's implantation

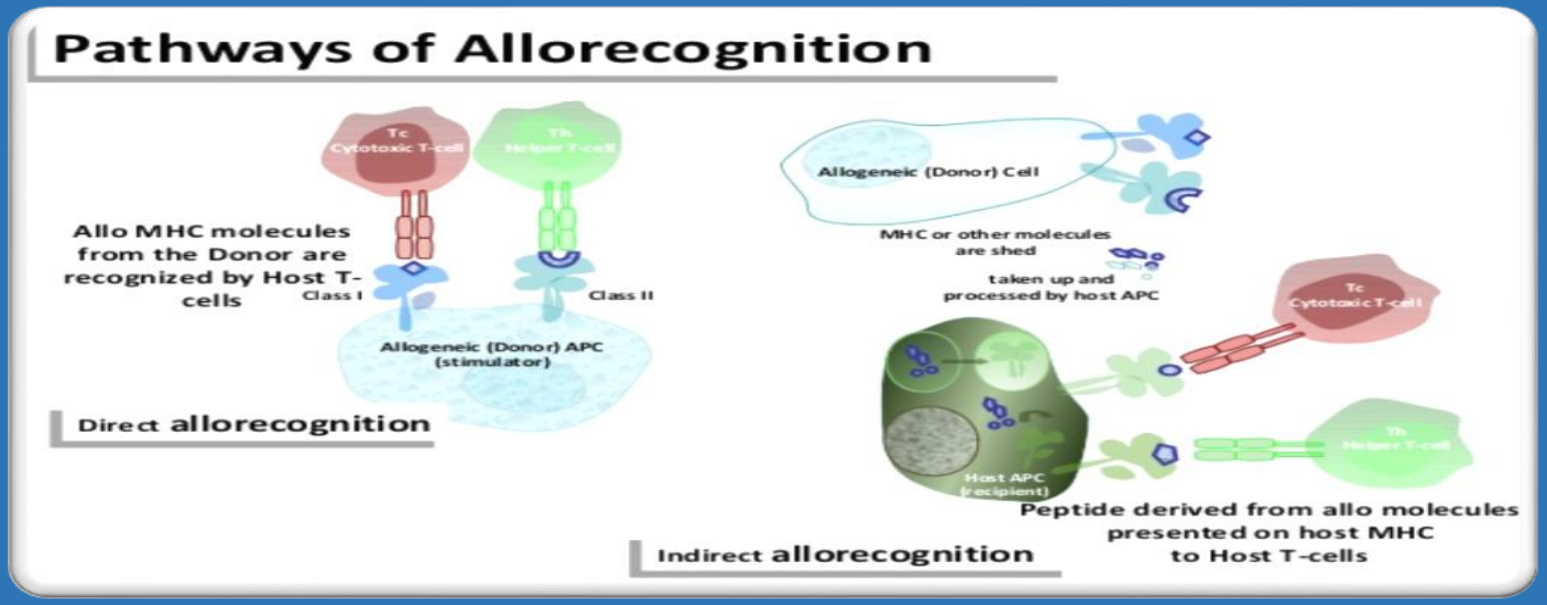
distinctiveness of fetal/maternal interactions

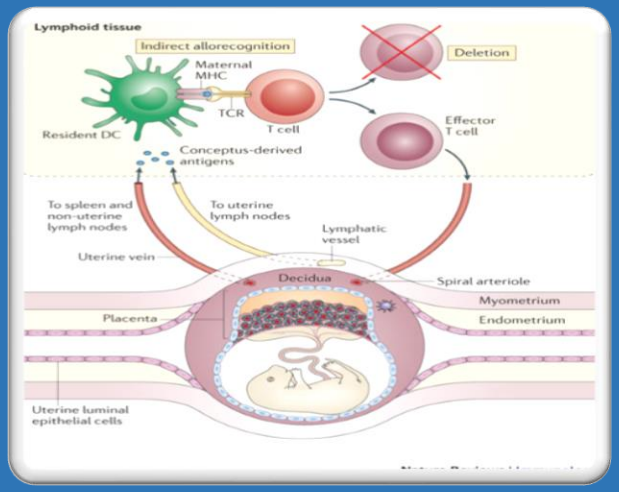
Allorecognition pathways

MHC and other molecules' expression in trophoblast

Phenotypical and functional characteristics of the decidual cells

The allorecognition pathways are different between Organ transplants and pregnancy





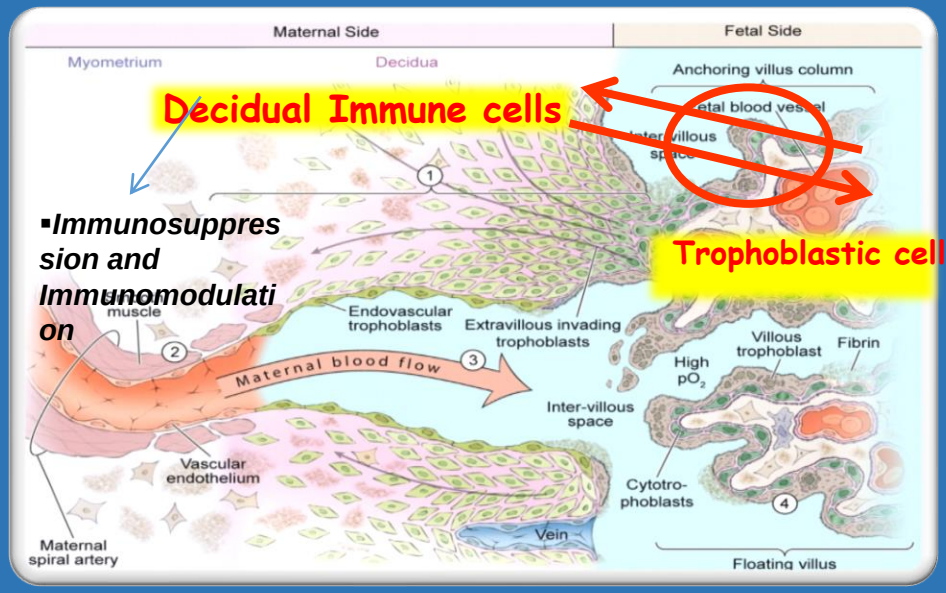
Unlike surgical organ transplants, the fetal 'allograft' is recognized by T cells exclusively via the **indirect allorecognition pathway** which is considered "minor"

NK cell-mediated allorecognition system in pregnancy

Fetal/maternal interface

The fetal-maternal interface is a unique microenvironment including three distinct components:

- fetal-derived trophoblast,
- maternal-derived decidual stromal cells, and
- immune cells



The trophoblast is the site of fetal antigen expression

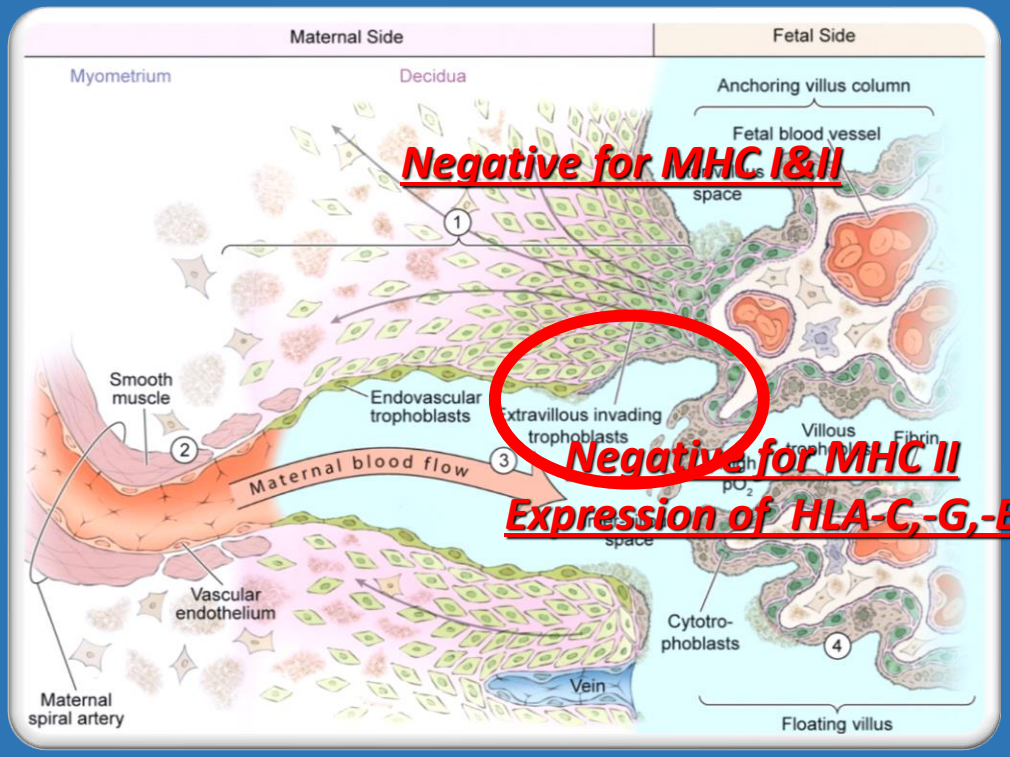
The maternal–fetal interface is the place of many complex connections between the mother's immune cells and the trophoblast cells

the establishment of immune tolerance

The unique expression of **HLA molecules** in trophoblasts and the interaction with their receptors in local immune cells are **key factors** for the establishment of immune tolerance

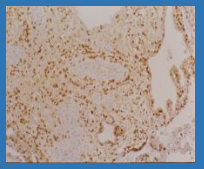
	cytotrophoblast		cyncytiotrophoblast
	villus	extra villus	
HLA-A, B,C	-----	C	-----
HLA-G,E,F	-----	G, E,F	-----
HLA-DR, DQ, DP	-----	-----	-----
MCP-CD46 (TLX)	+	+	+
R 80 K	+	+	+

HLA Class I expression: 7th week of gestation

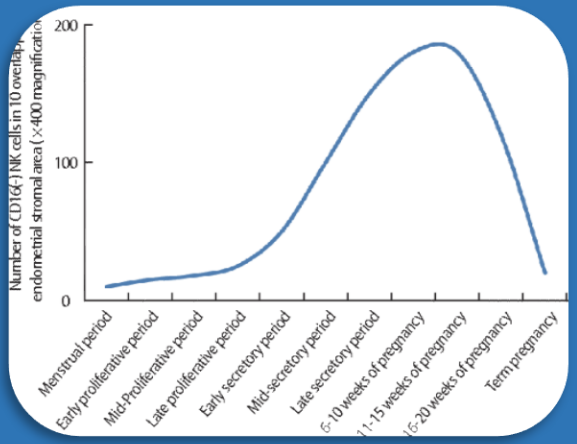


Only extravillous trophoblast cells express the non-polymorphic MHC class I molecules **HLA-G** (117 alleles), **HLA-E** (346 alleles) and **HLA-F** (59 alleles) and the more polymorphic **HLA-C** (7.672 alleles)

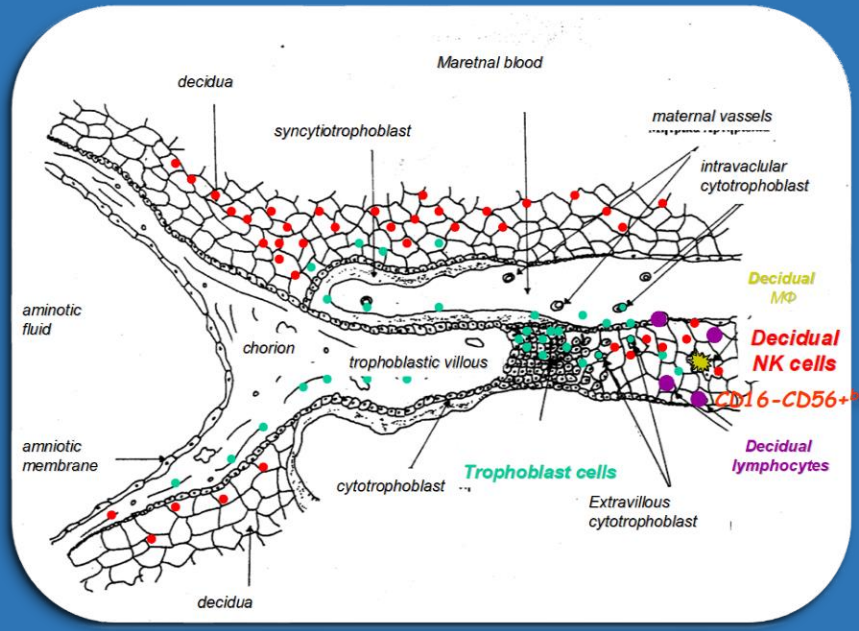
The Uterus is highly enriched in tissue-resident NK cells



increase during the secretor phase
when estrogens and progesterone prepare
the endometrium for a prospective pregnancy



they are present at high frequencies
in the decidua (dNK cells)
from the implantation stage
through the 1st trimester

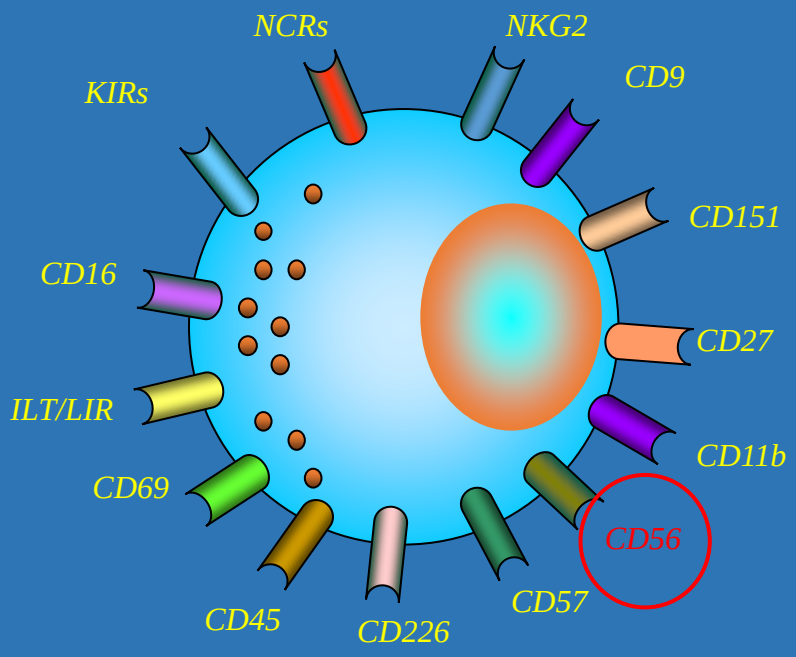


have direct contact with
trophoblastic cells

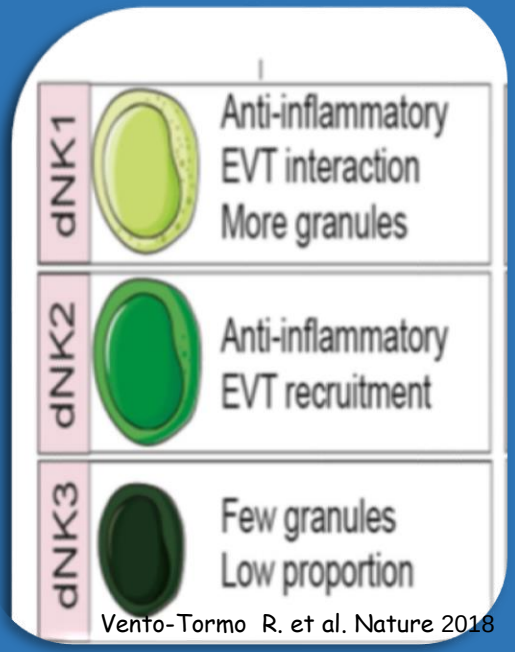
dNK

hold unique phenotypic/functional properties

$CD3^{-}CD16^{dim}CD56^{bright}$



three main dNK subsets



CD49a, CD9, CD39, CYP26A1, B4GALNT1, NKG2A, NKG2C, NKG2E, KIR2DS1, KIR2DS4, KIR2DL1, KIR2DL2, KIR2DL3, LILRB1

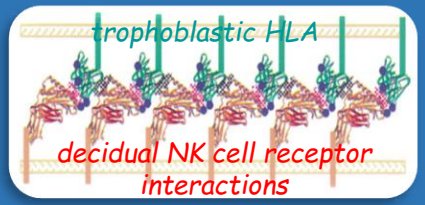
CD49a, CD9, ANXA1, ITGB2, NKG2A, NKG2C, NKG2E

CD49a, CD9,ITGB2, CD160, KLRB1, CD103,

dNK

play a key immunomodulatory role in early pregnancy and they are important for the establishment of normal pregnancy

NK cell-mediated allorecognition system in pregnancy



upon activation they produce:

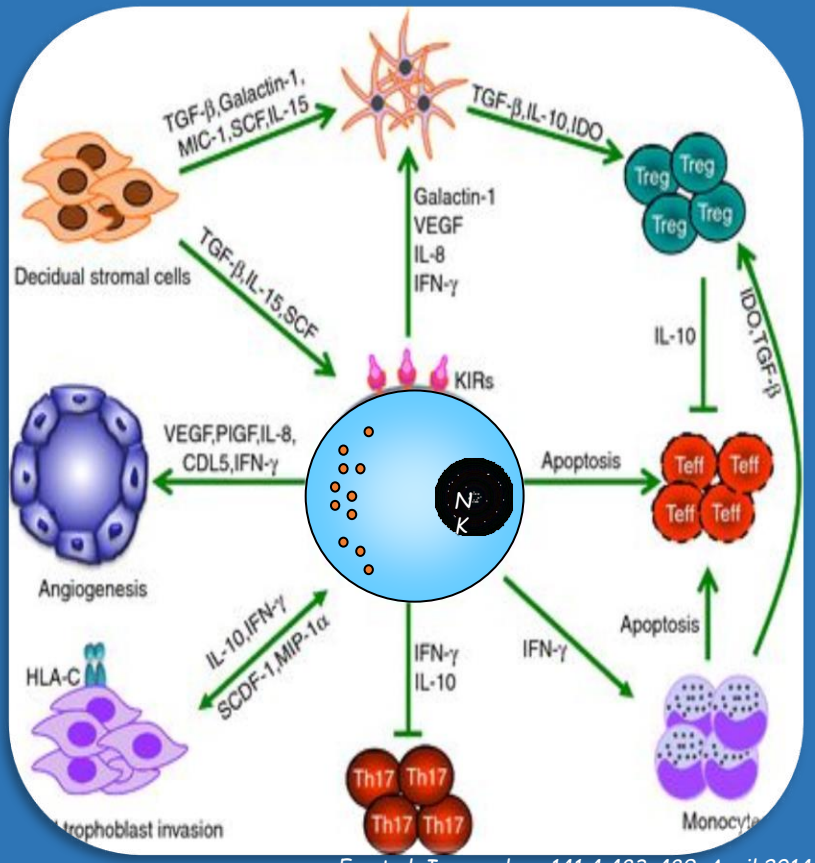
IFNy, perforin, angiogenetic factors, growth factors, Th2 cytokines

regulate

angiogenesis, uterine vascular remodelling, cell migration, trophoblast growth, differentiation, and invasion

although armed with both cytolytic mediators (perforin, fas/fas L)
do not kill trophoblast

in excessive Th1 (infection, inflammation) they become activated and cytotoxic



Fu et al, Immunology,141:4,483-489, April 2014

NK cell effector functions are regulated by the balance between activating and inhibitory signals transduced by

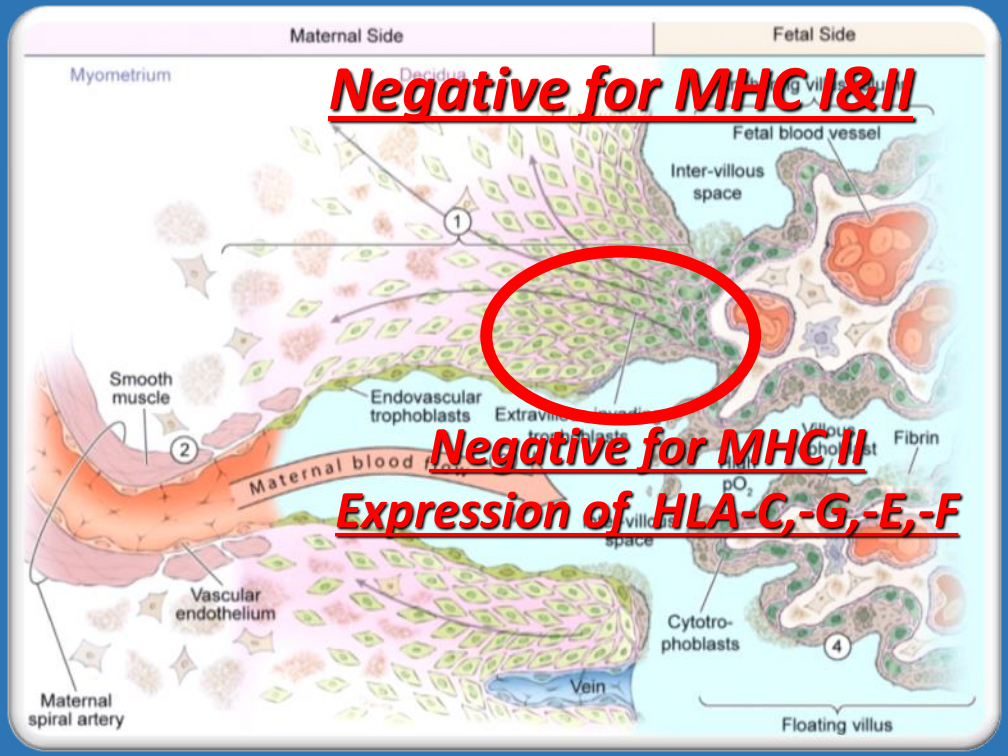
activating and inhibitory receptors

Family	Molecular structure	Receptors	Ligands
KIR	Immunoglobulin superfamily	KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DL4, KIR2DL1, KIR2DL2 KIR2DL3, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2, KIR3DL3	HLA-A,C, G, Bw4
NCR	Immunoglobulin superfamily	NKp44, NKp46, NKp30	Viral HA,….
ILT/LILR	Immunoglobulin superfamily	LILR-1,2,3,4,5,6a,6b,7,8	HLA class Ia (-G)
CD94/NKG2	C-type lectins	NKG2A/B, NKG2C,NKG2F NKG2E, NKG2H	HLA class Ib (-E)
NKG2D	C-type lectins	NKG2D	MICA,MICB ULBPs

*Through their **receptors**, dNK cells may recognize selected epitopes on*
HLA class I molecules
expressed on invading trophoblast

	cytotrophoblast		cyncytiotrophoblast
	villus	extra villus	
HLA-A, B,C	-----	C	-----
HLA-G,E,F	-----	G, E, F	-----
HLA-DR, DQ, DP	-----	-----	-----

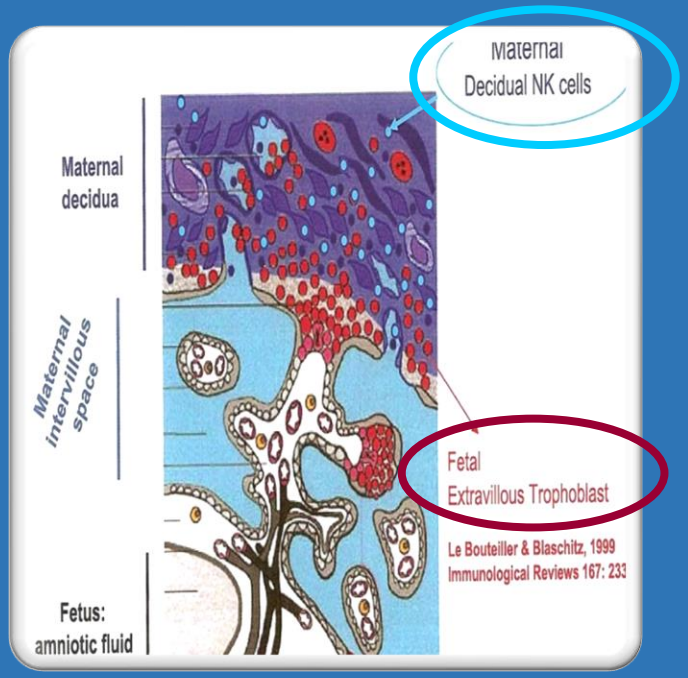
HLA Class I expression: 7th week of gestation



HLA-C, HLA-G, HLA-E, HLA-F
are the only HLA molecules expressed on extravillous trophoblast

Among the different NK receptors' interactions with their specific counterparts on trophoblast, the interactions between receptors of the ***KIR family and their ligands HLA-C*** *molecules appear to be those mainly involved in the function of an NK cell-mediated*

allorecognition system in pregnancy



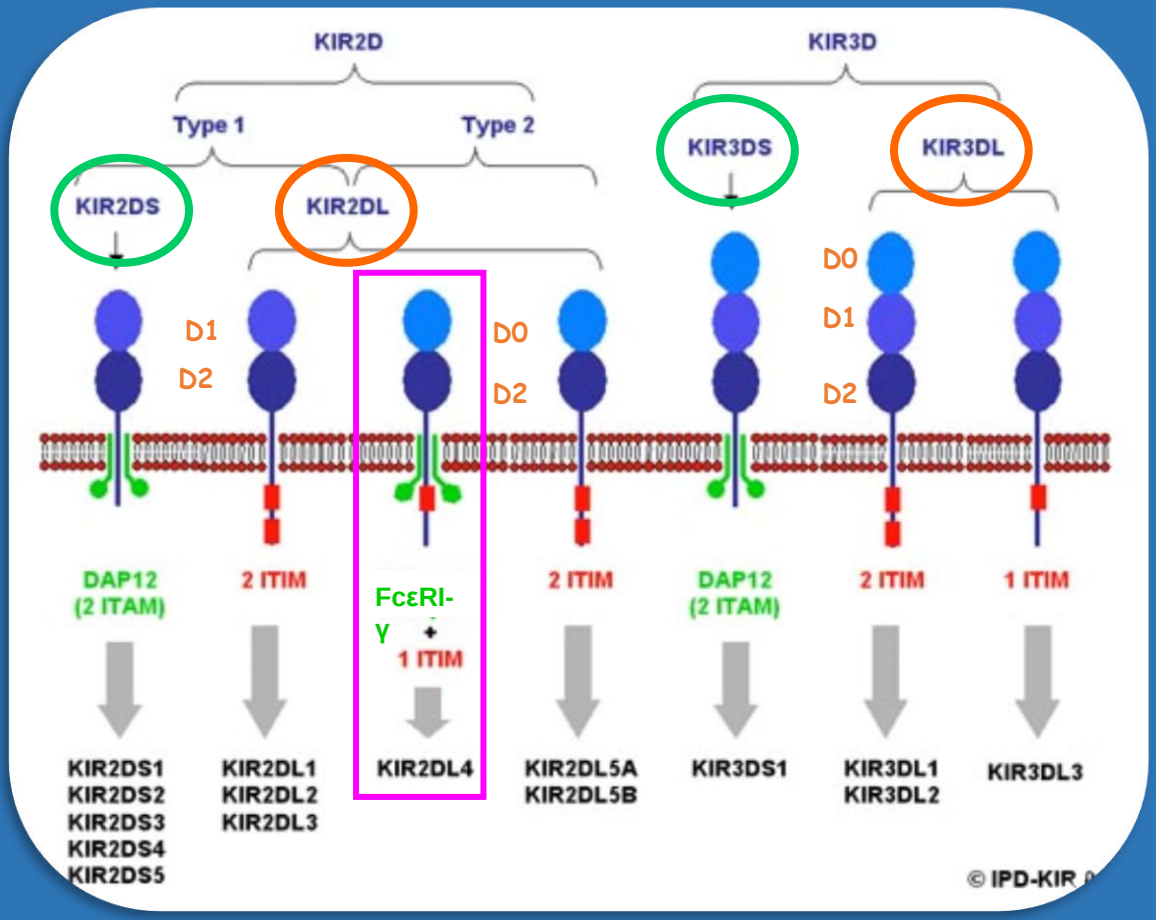
Given the differences in both the KIR repertoire and the HLA-C allotypes among unrelated individuals,

each pregnancy presents a different combination

of maternal KIR receptors on dNK and self and non-self HLA-C allotypes on trophoblast

This combination is expected to ensure the appropriate receptor–ligand interactions to favour pregnancy

Killer Immunoglobulin-like Receptors (KIR)



Transmembrane glycoproteins

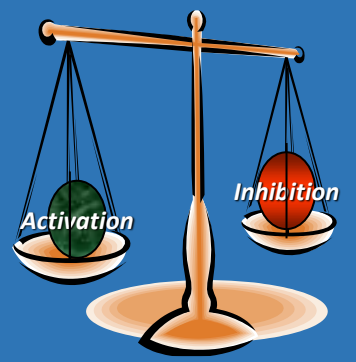
Activating→short (S) cytoplasmic tail

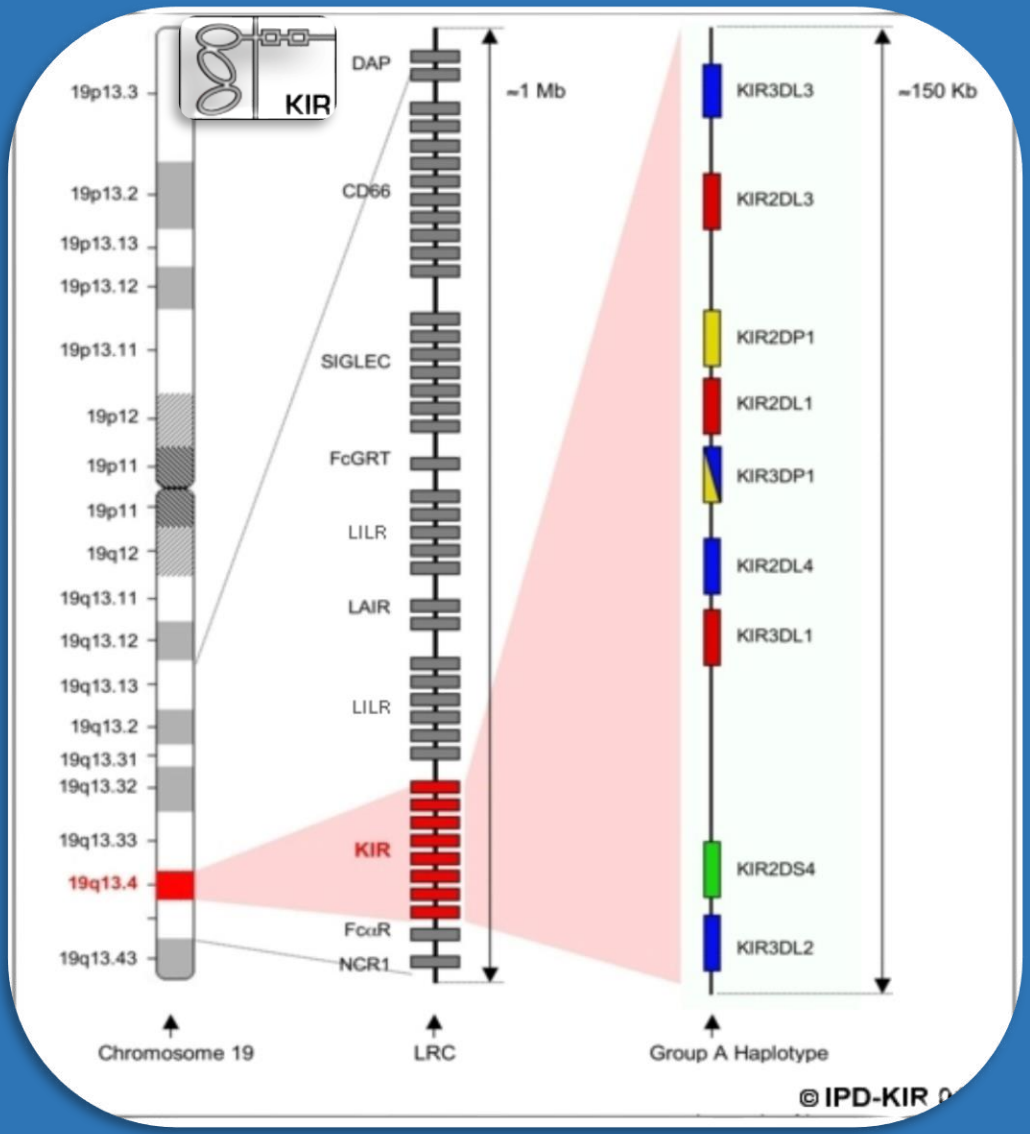
Inhibitory→long (L) cytoplasmic tail

Both activating and inhibitory receptors may co-expressed in the same cell

Activating receptors are effective only in the absence of ihnibitory receprots' action

INHIBITORY receptors regulate NK activation/action





KIR family consists of

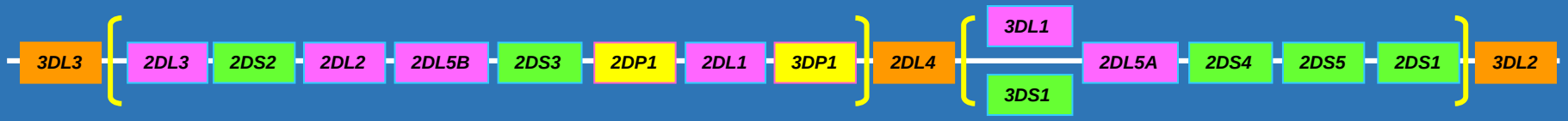
15 genes

KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1, KIR3DL2, KIR3DL3 και KIR3DS1

+

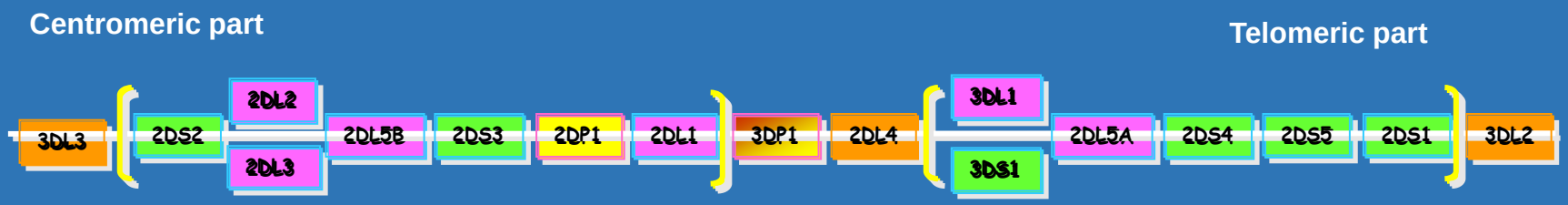
2 pseudogenes (KIR2DP1 and KIR3DP1)

encoded within a region of the LRC (Leukocyte Receptor Complex)

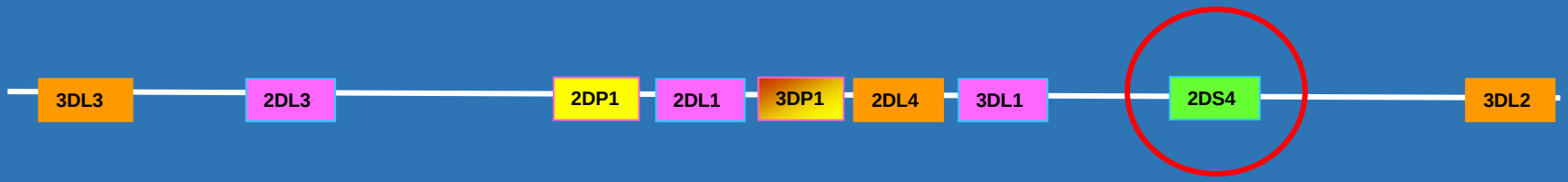


organized in haplotypes

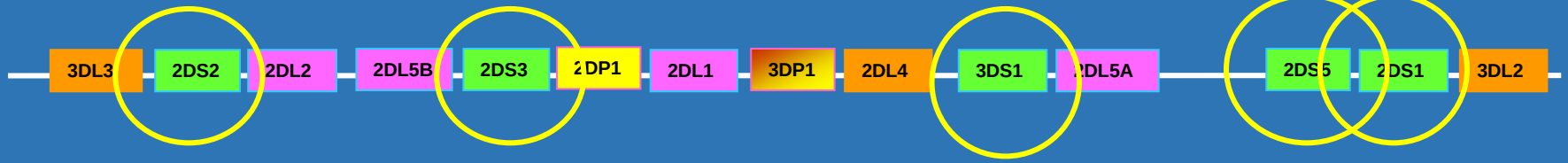
KIR genes are organised within the LRC into **haplotypes**, which have been shown to exhibit extensive variation in the number and type of KIR genes present



group A haplotype



group B haplotype



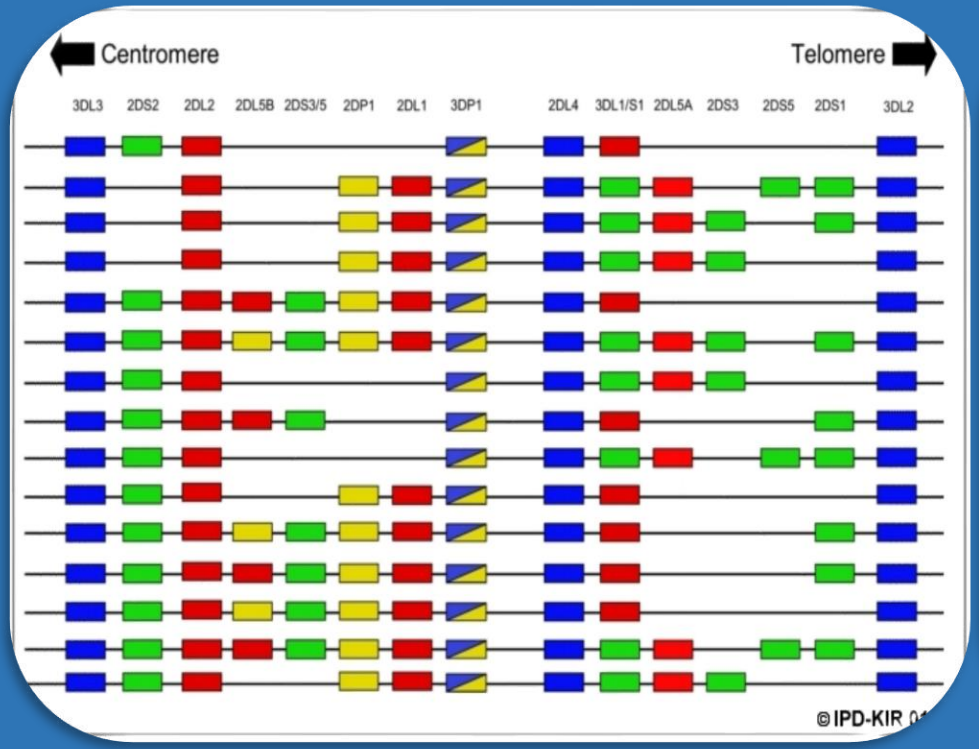
Framework genes | Activating KIR gene | Inhibitory KIR gene | Pseudogene

Uhrberg et al. 1997
Wilson et al. 2000
Vilches et al. 2000
Hsu et al. 2002

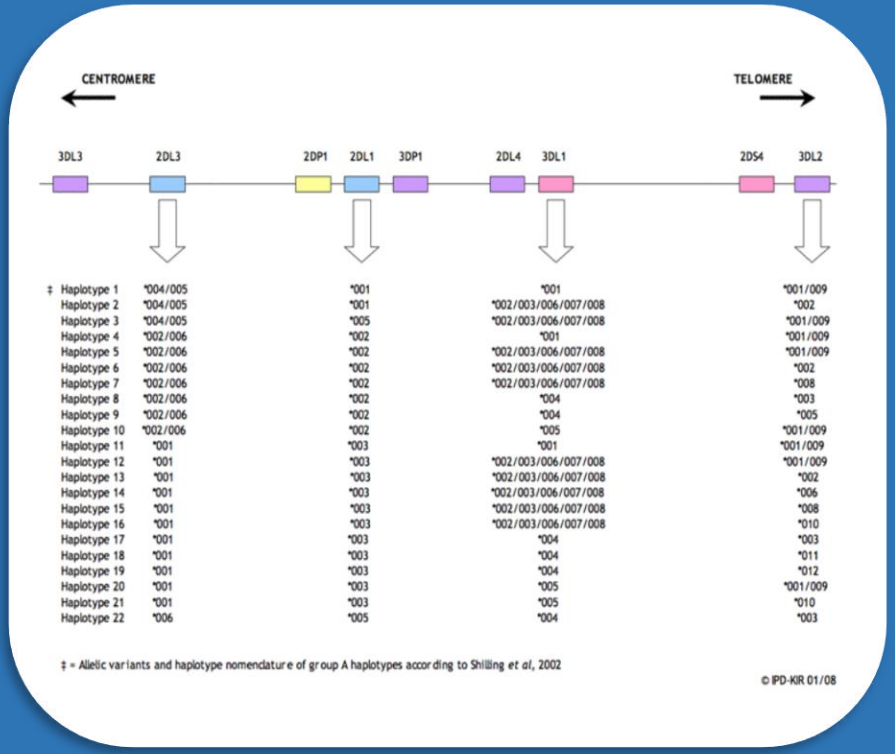
Within the human population,
KIR haplotypes and genotypes differ in their gene content,
and by allelic polymorphism at the individual KIR genes

KIR GENE DIVERSITY

variable number of genes depending on KIR haplotype



KIR ALLELIC POLYMORPHISMS



‡ Haplotype 1	*004/005	*001		*001/009
Haplotype 2	*004/005	*001	*002/003/006/007/008	*002
Haplotype 3	*004/005	*005	*002/003/006/007/008	*001/009
Haplotype 4	*002/006	*002	*001	*001/009
Haplotype 5	*002/006	*002	*002/003/006/007/008	*001/009
Haplotype 6	*002/006	*002	*002/003/006/007/008	*002
Haplotype 7	*002/006	*002	*002/003/006/007/008	*008
Haplotype 8	*002/006	*002	*004	*003
Haplotype 9	*002/006	*002	*004	*005
Haplotype 10	*002/006	*002	*005	*001/009
Haplotype 11	*001	*003	*001	*001/009
Haplotype 12	*001	*003	*002/003/006/007/008	*001/009
Haplotype 13	*001	*003	*002/003/006/007/008	*002
Haplotype 14	*001	*003	*002/003/006/007/008	*006
Haplotype 15	*001	*003	*002/003/006/007/008	*008
Haplotype 16	*001	*003	*002/003/006/007/008	*010
Haplotype 17	*001	*003	*004	*003
Haplotype 18	*001	*003	*004	*011
Haplotype 19	*001	*003	*004	*012
Haplotype 20	*001	*003	*005	*001/009
Haplotype 21	*001	*003	*005	*010
Haplotype 22	*006	*005	*004	*003

‡ = Allelic variants and haplotype nomenclature of group A haplotypes according to Shilling *et al*, 2002

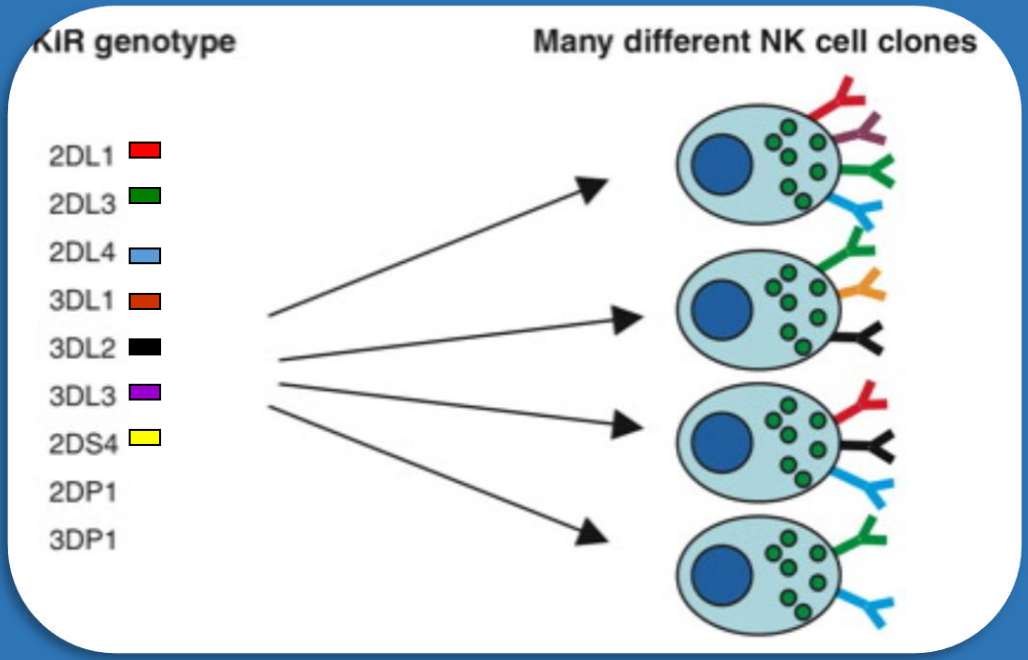
© IPD-KIR 01/08

KIR Expression

NK cells and subpopulations of T cells

Each NK cell clone of an individual does not express the entire set of KIR genes encoded in its genome

Possesses a diverse repertoire of NK cells with stochastically distributed KIR expression on their surface



Expression is under Transcriptional and post-transcriptional control

and is influenced by the presence or absence of HLA ligand

KIR ligands

HLA class I molecules

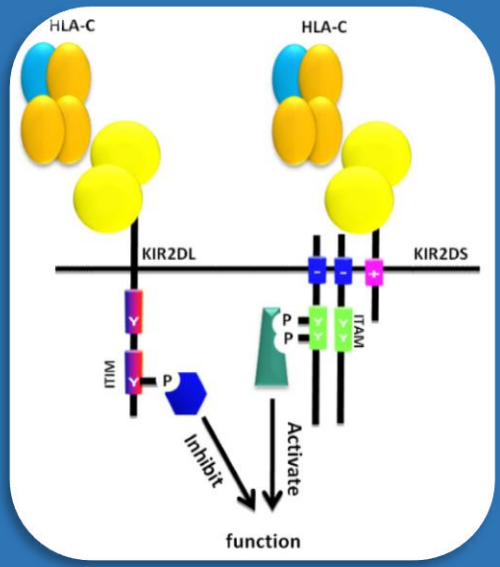
KIR	Ligands
2DL1	HLA C2 group
2DL2,3	HLA C1 group
2DS1	HLA C2 group
2DS2	?
2DS3,5	?
2DS4	HLA-A*11 and some HLA-C1+C2, HLA-F
2DL4	HLA-G
2DL5	?
3DL1	HLA-Bw4
3DL2	HLA-A3, A11, HLA-F…?
3DS1	HLA-B (Bw4) ?

KIR2DL1
KIR2DS1 → HLA-C2

KIR2DL2/3 → HLA-C1, B*46:01, 73:01
HLA-C2 low affinity

KIR3DL1 → HLA-Bw4

HLA-C are the ligands for most of the KIRs



HLA-C Asn^{80}

*HLA C1 group: C*01, C*03, C*07, C*08, C*12, C*13, C*14, C*16:01/4*

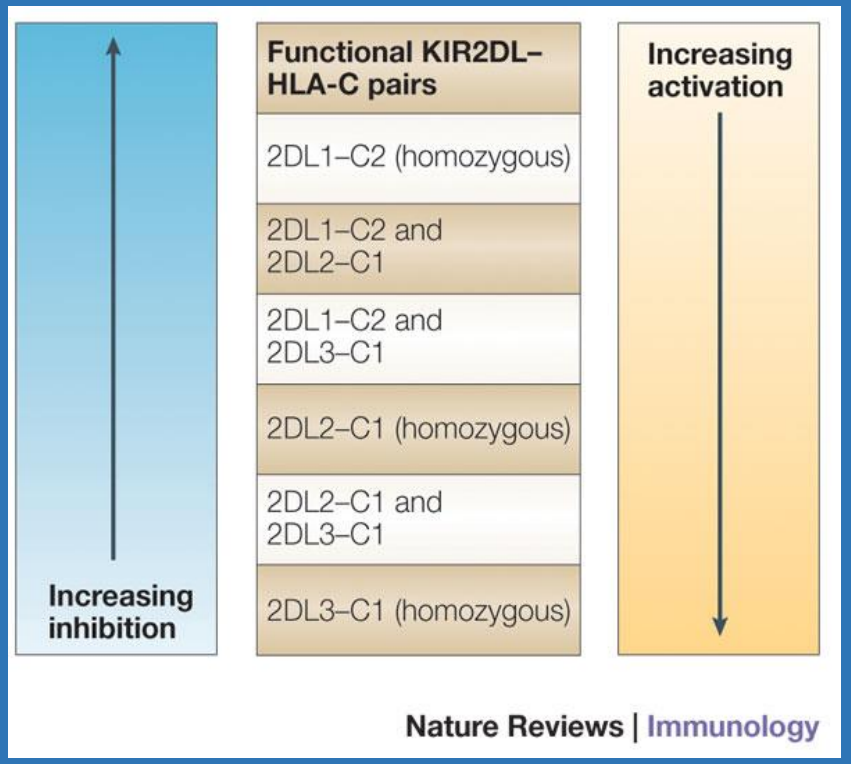
HLA-C Lys^{80}

*HLA C2 group: C*02, C*04, C*05, C*06, C*15, C*16:02, C*17, C*18*

Hierarchy

The activating KIRs bind their ligands with lower affinity than that of inhibitory receptors

Inhibitory receptor	HLA–C ligand		Affinity of interaction
KIR2DL1	Lysine 80	C2	Strong
KIR2DL2	Asparagine 80	C1	Intermediate
KIR2DL3	Asparagine 80	C1	Weak



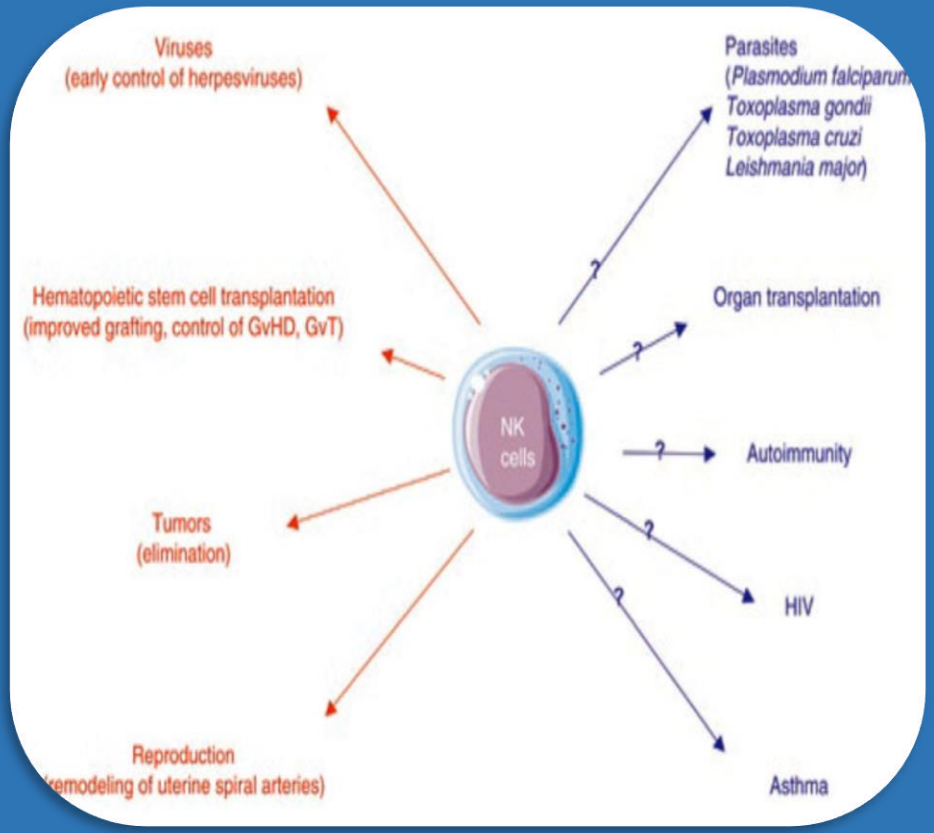
P. Parham, Nat Rev Immunol 2005

KIR2DL2- HLA-C2 weakly

KIR2DS1-HLA-C2

KIR / HLA-C interactions seem to play a role in

Infectious diseases
Autoimmune/inflammatory disorders
Cancer
and alloimmune responses such as
Transplantation and
Reproduction

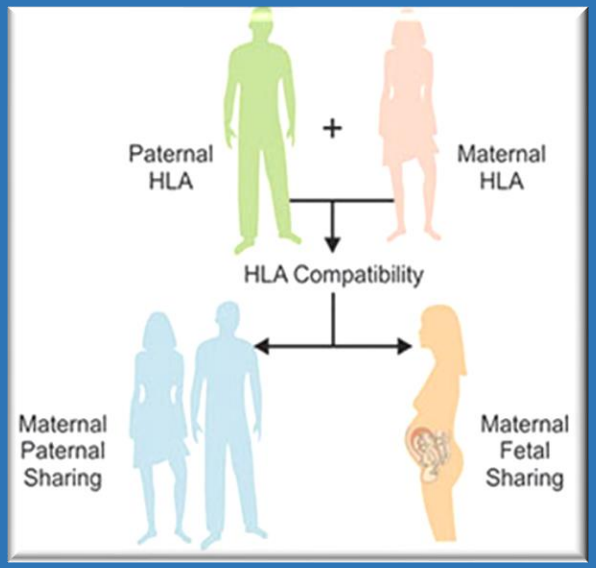


HLA involvement in reproductive success

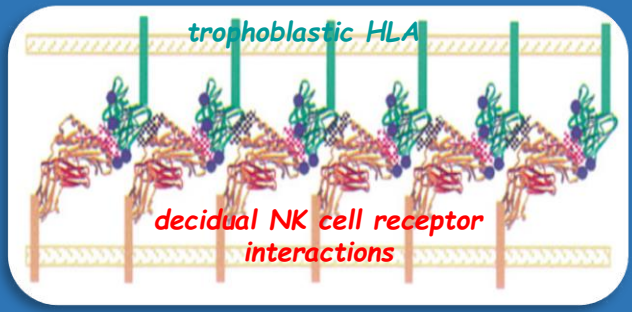
Specific HLA antigens/alleles

HLA couple sharing

HLA mother-fetus sharing



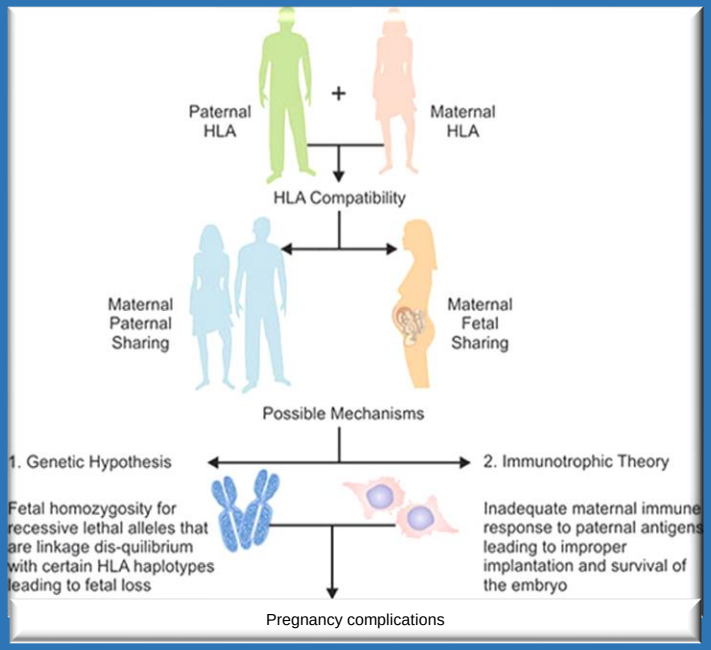
HLA-C/KIR interactions



Have been associated with pregnancy outcome and risk of complications

The genetic hypothesis

Reproductive failure is due to homozygosity for recessive lethal alleles that are in linkage disequilibrium with specific HLA haplotypes

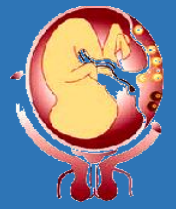


The immunological hypothesis

HLA Compatible embryo

Insufficient antigenic stimulus for maternal response to enhance pregnancy

HLA Incompatible embryo



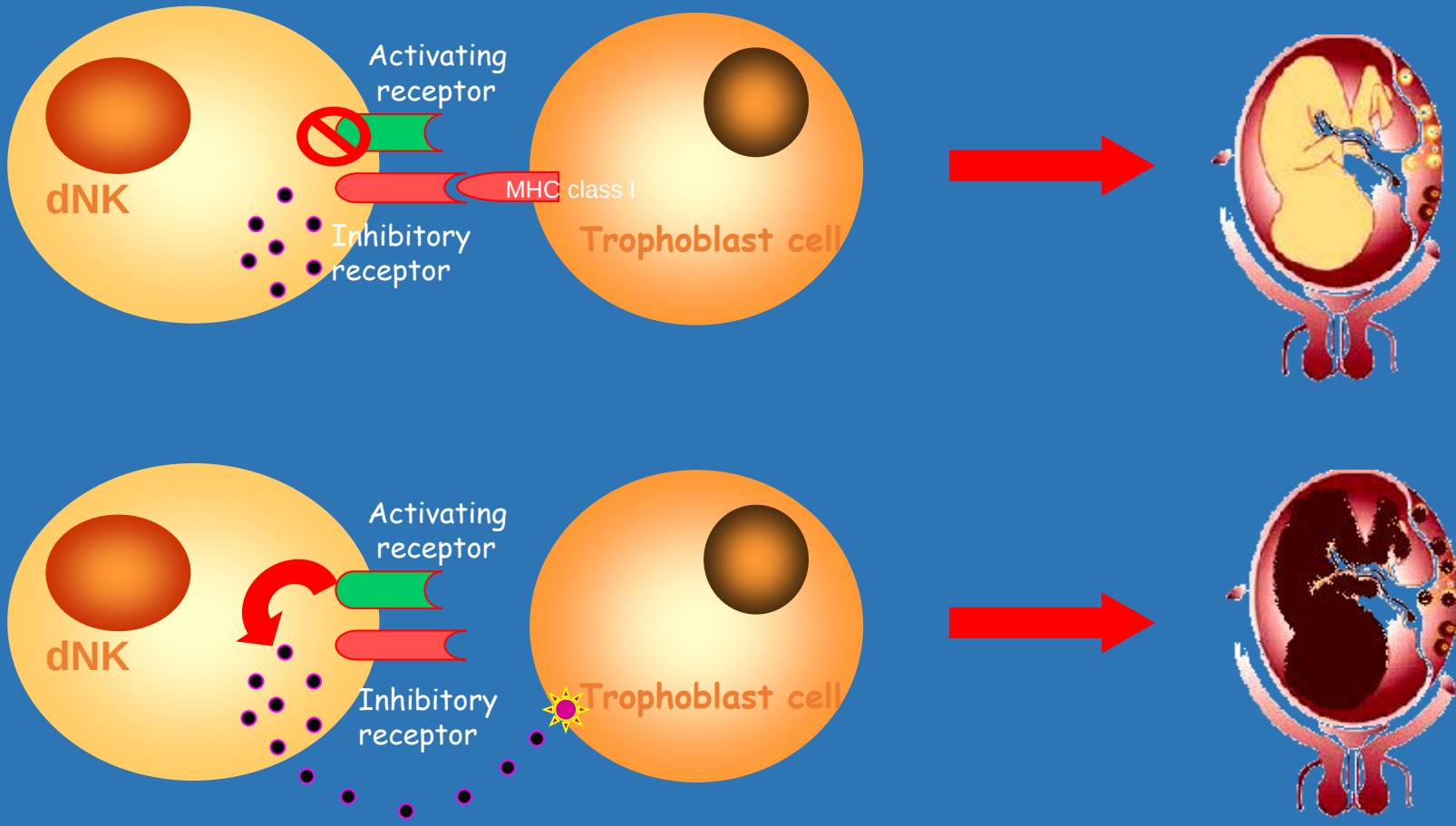
Sufficient antigenic stimulus for maternal response to enhance pregnancy

KIR/HLA-C allorecognition system in pregnancy

(Varla-Leftherioti, 2004)

Hypothesis:

If trophoblastic HLA molecules are not recognized by dNK cell appropriate receptors, the functions of the NK cells can be detrimental for trophoblast



HLA involvement in reproductive success

HLA sharing story

'70s

Increased HLA sharing
in couples with recurrent spontaneous abortions (RSA)
Komlos L et al, 1977

→ large number of studies with **controversial results**

HLA in RM

↑

Kolmos et al	1977
Gerenceret al	1978
Reznikoff-Etievant et al	1984
Unander et al	1983
.....	
Aruna et al.	2011
Thomsen et al.	2021

↓

Oskenberg et al	1983
Mowbray et al	1983
Thomas et al	1985
Christiansen et al	1989
.....	

Kwak-Kim et al 1990

Kwak et al 1991
Ober et al 1991,1993

DQA1
DQA1*0505 ?

DQA1
DQA1*0505

11th IHWC (Yokohama 1991)

13th IHWC (Victoria 2001)

14th IHWC (Melbourne 2005)

15th IHWC (Bouzios 2008)

HLA in RIF

↑

Weckstein LN et al , 1991
Matsuyama T, et al , 1992

Balasch J, et al, 1993

Carp et al, 1994

Ho HN, et al, 1994
Creus M, et al, 1998

* Ober C, et al, 1993

* Jin K, et al , 1995

↓

Martin-Villa JM, et al,, 1993

* Check JH, et al, 2001

** DQA1*05:05 sharing*

HLA- C group sharing

HLA in RM

Increased DQA1*05:05 sharing in RM couples with women having autoimmune disturbances

Keramitsoglou et al., 2004
(86 RSA couples)

Controls	6/36 16.6%
Auto RPL	14/58 24.3%

Varla-Leftherioti M et al 2007
(108 RSA couples)

Controls	48/182 26.37%
Auto RPL	16/68 24,6%

Varla-Leftherioti M et al 2010
(185 RSA couples)

DQA1*05:05 sharing does not characterize neither allo- nor auto-immune aborters

HLA in RIF

In RIF couples no difference were found in allele sharing between partners compared to the controls

31 RIF and **31 Controls** HLA-A*, -B*, -C*, -DRB1*, -DQA1*, -DQB1* typing

	0	1	2	3	4	5	6	7	8	9	10-12	≥3
Controls	12.9	22.5	22.5	22.5	16.2	3.2	3.2					45%
IVF	18.7	31.2	25	6.25	6.25	6.25	6.25					25%

DQA1*05:05

Controls	5,5%
RIF	10%

No of failures:
all: 5.7
DQ compatible: 7.6

HLA-C

HLA and KIR typing in couples with subfertility problems
Th. Keramitsoglou, Ch. Tsekoura, B. Geladakis, M. Varla-Leftherioti
LISBON EFI 2019

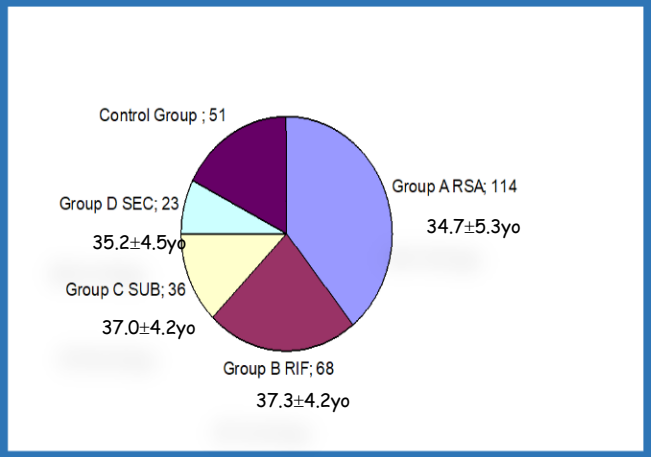
33rd EUROPEAN IMMUNOGENETICS AND HISTOCOMPATIBILITY CONFERENCE
FUNCTIONAL IMMUNOGENETICS: THE HISTORICAL CHALLENGE

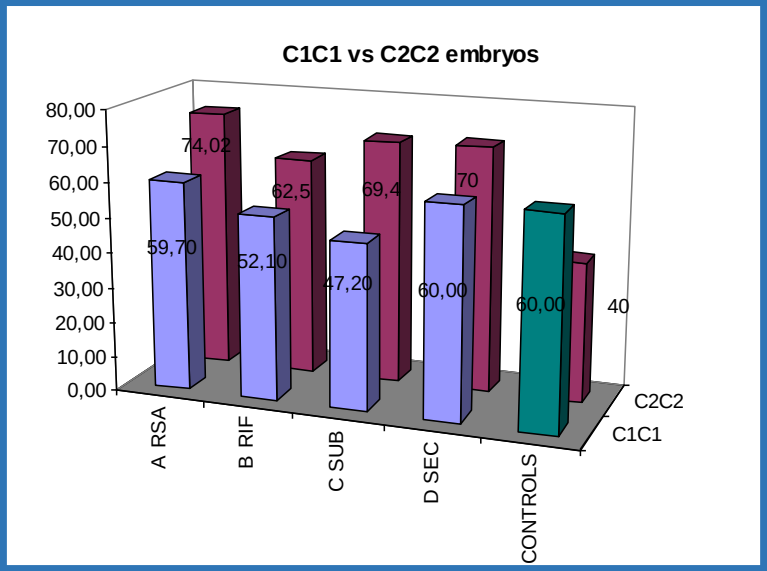
260 subfertile couples

Embryos with estimated HLA-C2C2 homozygocity clearly predominated over HLA-C1C1 embryos in all patients' groups

RSA:C1C1 59.7% vs *C2C2 74.02%*,
RIF:52.1% vs *62.5%*,
SUB:47.2% vs *69.4%*,
SEC:60% vs *70%*,

but not in the group of fertile couples (*C1C1: 60%* vs C2C2 40%)





Preeclampsia

	Uncomplicated (n=451)						
	Observed (number of matches)			Expected (number of matches)			P-value*
	1	2		1	2		
HLA-A	86.0%	14.0%		88.2%	11.8%		0.244
HLA-B	91.6%	8.4%		92.9%	7.1%		0.271
HLA-C	85.8%	14.2%		86.9%	13.1%		0.485
HLA-DRB1	88.2%	11.8%		90.7%	9.3%		0.075
HLA-DQB1	81.4%	18.6%		83.1%	16.9%		0.314
Class I	3	>3		3	>3		
	74%	25.9%		73.9%	26.1%		0.915
Class II	2	>2		2	>2		
	78.7%	21.3%		79.4%	20.6%		0.727
Total	5	6	>6	5	6	>6	
	59.2%	25.1%	15.7%	59.2%	27.7%	13.1%	0.166

	Preeclampsia (n=77)							P-value* Uncomplicated vs Preeclampsia (observed only)
	Observed (number of matches)			Expected (number of matches)			P-value*	
	1	2		1	2			
HLA-A	85.7%	14.3%		88.3%	11.7%		1.000	1.000
HLA-B	87.0%	13.0%		93.5%	6.5%		0.098	0.097
HLA-C	77.9%	22.1%		89.6%	10.4%		0.007	0.025
HLA-DRB1	87.0%	13.0%		93.5%	6.5%		0.326	0.710
HLA-DQB1	77.9%	22.1%		84.4%	15.6%		1.000	0.382
Class I	3	>3		3	>3			
	64.9%	35.1%		75.3%	24.7%		0.240	0.038
Class II	2	>2		2	>2			
	75.3%	24.7%		81.8%	18.2%		1.000	0.366
Total	5	6	>6	5	6	>6		
	48.0%	27.3%	24.7%	63.6%	24.7%	11.7%	0.012	0.021

Preeclamptic pregnancies show a tendency of higher maternal-fetal HLA-C, HLA class I, and total HLA matching, compared to uncomplicated pregnancies

van 't Hof LJ, et al. Maternal-Fetal HLA Compatibility in Uncomplicated and Preeclamptic Naturally Conceived Pregnancies. Front. Immunol. 12:673131 (2021)

Specific HLA antigens/alleles

In a recent large case-control study it was found that the HLA-DRB1*07 allele was highly significantly associated to RM

Thomsen C.K. et al. Journal of Reproductive Immunology 145 (2021)

Table 1
Frequencies of HLA-DRB1 alleles in recurrent pregnancy loss (RPL) patients and bone marrow donor controls.

HLA-DRB1*	RPL (n = 2156)	controls (n = 4132)	OR (95 % CI)	P; Pc
01	10.76	11.08	0.97 (0.82–1.14)	0.7
03	13.08	13.53	0.96 (0.83–1.12)	0.6
04	17.16	16.36	1.06 (0.92–1.22)	0.4
07	12.29	9.80	1.29 (1.09–1.52)	< 0.0025; 0.03
08	3.15	3.75	0.84 (0.63–1.12)	0.2
09	1.07	0.85	1.26 (0.74–2.14)	0.4
10	0.70	0.56	1.25 (0.65–2.40)	0.5
11	7.42	6.97	1.07 (0.88–1.31)	0.5
12	2.46	2.54	0.97 (0.69–1.35)	0.8
13	12.76	13.75	0.92 (0.79–1.07)	0.3
14	2.27	2.23	1.02 (0.72–1.45)	0.9
15	16.14	17.52	0.91 (0.79–1.40)	0.2
16	0.83	1.06	0.78 (0.45–1.36)	0.4

Pc: p-values adjusted for number of comparisons (n = 13).

1078 Caucasian women with RM
2066 controls

Table 2. Impact of maternal HY-restricting HLA class II alleles on the chance of a subsequent live birth in secondary recurrent miscarriage patients

HLA class II characteristics	Sex of child born prior to the miscarriages						Chance of live birth		
	♂ $n = 166$			♀ $n = 120$			♂ compared with ♀ prior to miscarriages		
	Total n	Live birth n	%	Total n	Live birth n	%	OR*	95% CI	P-value
HLA-DRB1*15	50	22	44	35	29	83	0.16	0.06–0.46	0.001
HLA-DQB1*0501/0502	38	15	40	24	20	84	0.14	0.04–0.50	0.003
HLA-DQB1*0503	10	8	80	7	6	86	0.35	0.02–7.9	0.513
HLA DRB3*0301	9	5	56	13	11	85	0.23	0.03–1.68	0.14
HY-restricting HLA class II	89	39	44	62	51	82	0.17	0.08–0.39	0.0001
No HY-restricting HLA class II	77	51	66	58	40	69	0.90	0.42–1.87	0.76

*Adjusted for number of previous miscarriages.

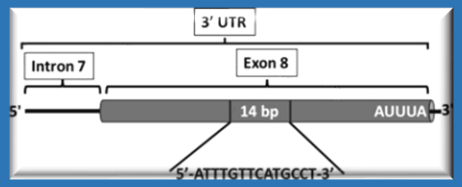
A prospective study (n=358) provided evidence that women with secondary RM after the birth of a boy have a significantly lower (22%) subsequent live birth rate when they carried one of DRB1*15:01; -DQB1*05:01/05:02 and -DRB3*03:01 alleles, known to predispose to clinically relevant anti-HY immune reactions

Nielsen HS, et al. *Hum Mol Genet* (2009)

HLA-G

Abnormal sHLA-G expression and HLA-G polymorphisms are associated with pregnancy complications such as preeclampsia, recurrent miscarriage (RM), and recurrent implantation failure (RIF)

HLA-G 14-bp insertion/deletion polymorphic variation was associated with RM risk in patients with three or more miscarriages



Fan, W., Li, S., Huang, Z. *et al.* *J Assist Reprod Genet* (2014).

The presence of sHLA-G in the embryo culture medium favored higher implantation rate and pregnancy rate

Ziru Niu et al. Reproductive BioMedicine Online (2017)

- HLA-G 14bp ins/ins homozygous genotype or ins variant was associated with a higher risk of RIF in the Caucasian population

- The maternal HLA-G*010101 and paternal HLA-G*010102 alleles are associated with RIF risk compared to other alleles

Hu L, et al. Front. Immunol (2022)

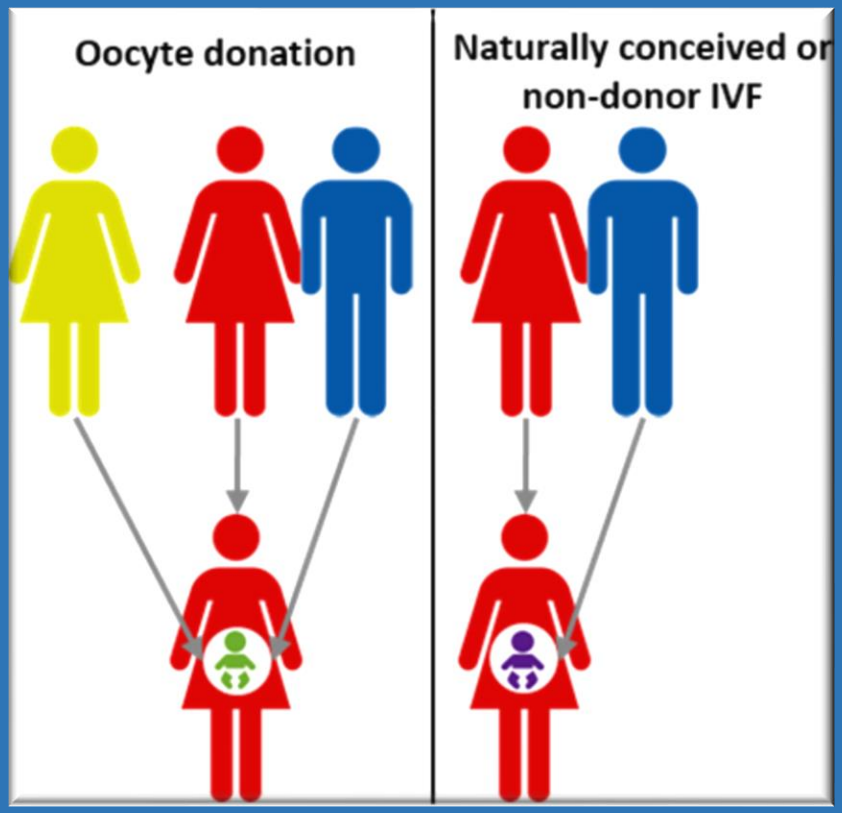
Table 1
HLA associations in RM.

	Author	Study Design	Cases		Control subjects		Ethnicity	HLA biomarker	Study findings
			N	Definition	*N*	Definition			Significant
Classical HLA I									
1	Faridi et al. [8]	Case control	177	⩾3 PRM	200	⩾2 uncomplicated live births	Ethnically matched	C1, C2 alleles in couples (allelic)	–
2	Hiby et al. [10]	Case control	162	⩾3 PRM, first (92%) and second trimester, same partner	269	1 live birth	NR	C1, C2 alleles in couples (allelic)	C2
3	Christiansen et al. [61]	Case control	70	⩾3 RM (20 PRM, 15 SRM), before 28th gestational week	60	⩾2 live births	Caucasian	C1, C2 alleles in couples (phenotypic)	–
Classical HLA II									
4	Aruna et al. [6]	Case control	56	143 couples with ⩾2 RM (130 PRM, 13 SRM) and 56 couples with ⩾3 RM	140	⩾1 live birth	Ethnically matched	DRB1, DQA, DQB (allelic)	DQB1*03:03:02[a] DQB1*03:03:03↓[b]
5	Kruse et al. [5] (study II)	Case control	354	⩾3 RM (212 PRM, 142 SRM), 20–45 years	202	⩾1 live birth	Caucasian	DRB1, DQA1, DQB1 (phenotypic)	DRB1*04[b], DRB1*13[b], DRB1*14[b], DQA1*01:03[b], DQB1*03:02[b], DQB1*06:03/06:04[b] (study II)
6	Takakuwa et al. [65]	Case control	93	⩾3 RM (79 PRM, 14 SRM) first trimester, same partner	115	⩾2 term deliveries	Japanese	DRB1 (phenotypic)	DRB1*15:02
7	Sasaki et al. [57]	Case control	27	⩾3 RM, first trimester	22	⩾2 term deliveries	NR	DRB1 (phenotypic)	DRB1*04
8	Takakuwa et al. [60]	Case control	30	⩾3 PRM, first trimester, same partner	30	⩾2 term deliveries		DPB (phenotypic)	DPB*04[b], DPB*04:02[b]
9	Bellingard et al. [62]	Case control	10	⩾3 PRM, mean age 33.9 years	21	⩾2 live births	NR	DRB1 (allelic and phenotypic)	–
10	Dizon-Townson et al. [45]	Case control	51	⩾3 RM, consecutive	43	⩾7 live births	Caucasian	DQA1 (allelic)	–
11	Takakuwa et al. [63]	Case control	22	⩾3 RM, same partner, first trimester	20	⩾2 term deliveries	NR	DQB1 (allelic and phenotypic)	–
Non-classical HLA II									
12	Christiansen et al. [16]	Case control	339	⩾3 RM (154 PRM, 185 SRM), median age at referral 32–33 years	125	⩾2 uncomplicated live births	NR	HLA-G (exon 8)	G14 bp ins/ins
13	Vargas et al. [14]	Case control (matched age, socioeconomic)	60	⩾3 PRM (clinically verified), before 20th gestational week, same partner, mean age at miscarriage 26.4 years	68	⩾2 live births	Ethno-geographically matched	HLA-G (exon 2, 3, 8) (allelic)	HLA-G 01:01A↓
14	Zhu et al. [46]	Case control	51	⩾3 RM	251	⩾1 live birth	NR	HLA-G (exon 8)	–
15	Suryanarayana et al. [51]	Case control	169	⩾3 PRM, first trimester	92	⩾1 uncomplicated	Ethnically	HLA-G (exon 2	–
16	Xue et al. [17]	Case control	24	⩾3 RM					
17	Yan et al. [47]	Case control	79	⩾3 RM					
18	Yan et al. [47]	Case control	69	⩾3 RM					
19	Abbas et al. [66]	Case control	120	⩾3 PRM					
20	Tripathi et al. [67]	Case control	120	⩾3 PRM					
21	Pfeiffer et al. [15]	Case control	78	⩾3 RM (56 PRM, 22 SRM), same part 42 years					

Although the present systematic review and meta-analysis demonstrates that specific HLA alleles and HLA sharing are associated with RM, a high degree of bias was present and therefore observed results should be interpreted carefully

HLA associations and HLA sharing in recurrent miscarriage: A systematic review and meta-analysis

T. Meuleman et al. / Human Immunology 76 (2015) 362–373



In Oocyte Donation pregnancy, the fetus may be completely allogeneic to the mother

Possibly, the allogeneic nature of the fetus in OD pregnancies plays a role in the development of pregnancy complications, such as

premature birth,
low birthweight,
bleeding complications, and
hypertensive disorders

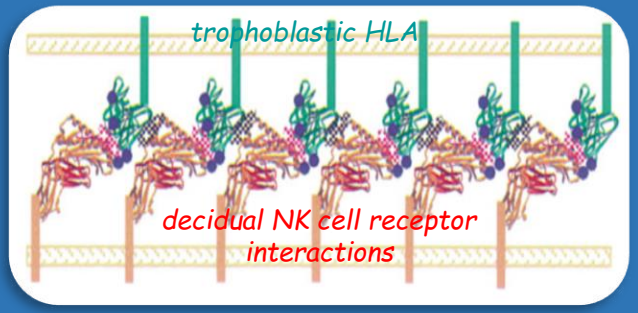
A significant higher level of HLA matching between mother and child in successful and uncomplicated OD pregnancies than expected by chance

Lashley et al. Journal of Reproductive Immunology (2015)

A higher number of HLA class II mismatches, and specifically HLA-DR mismatches, is associated with a higher chance of developing preeclampsia in OD pregnancies

van Bentem K. Journal of Reproductive Immunology (2019)

HLA-C/KIR interactions



The first positive association of recurrent miscarriages with KIR repertoire was presented by Varla-Leftherioti et al

KIR and CD94/NKG in fertile and alloRSA couples

(Varla-Leftherioti et al, Am J Reprod Immunol 2003:49:183-91)

Studies of association between KIR and KIR/HLA-C combinations and RM are conflicting

Table 1 Basic research studies showing the associations between KIR and HLA and recurrent miscarriage

Reference	KIR (and HLA) implicated	Type of experiment/objective	Conclusions
[40]	Inhibitory KIRs (2DL1, 2DL2, and 2DL3)	26 childless couples with ≥2 abortions and 26 control couples. KIR genotyping	Some alloimmune abortions may occur when the MHC class I molecules on trophoblasts are recognized by decidual NK cells lacking appropriate inhibitory KIR receptors that would stop activating signals.
[41]	No association	51 women with unexplained recurrent spontaneous abortions consecutively referred/55 controls. KIR genotyping.	The data provide little evidence that KIR polymorphism plays a role in predisposition to recurrent spontaneous abortions.
[42]	Inhibitory KIRs (in particular 2DL2)	Cohort of 30 fertile couples (without previous abortions)/139 healthy controls/88 couples with ≥3 recurrent spontaneous abortions. KIR genotyping	The balance between inhibitory and activating receptors present in natural killer cells is inclined toward an activating state that may contribute to pregnancy loss.
[43]	Activating KIRs (in particular 2DS1). KIR2DS1 in the absence of KIR2DL1/HLA-C2.	73 pairs of childless couples with ≥3 abortions characterized as unexplained and 68 pairs of healthy control couples. KIR genotyping and HLA-C groups C1/C2 identification.	A decrease in the ligands for inhibitory KIRs could potentially lower the threshold for NK cell activation, mediated through activating receptors, thereby contributing to the pathogenesis of recurrent spontaneous abortion.
[44]	KIR2DS1	Male ($n = 67$) and female ($n = 95$) partners of couples with ≥3 spontaneous miscarriages/269 controls (women primiparae, no miscarriages, or ectopic pregnancies). KIR genotyping and HLA-C groups' identification.	The findings support the idea that successful placentation depends on the correct balance of uNK cell inhibition and activation in response to trophoblasts.
[45]	Activating KIRs	68 patient couples with recurrent miscarriage and 68 control fertile couples. KIR genotyping	Recurrent miscarriage could be associated with NK cell activation mediated by a profile rich in activating KIR genes.
[46]	KIR2DL1/HLA-C2 KIR2DS2/HLA-C1	177 couples with recurrent miscarriages (primary aborters, no live births) and 200 healthy couples (at least two live births and with no history of miscarriage, preeclampsia, ectopic pregnancy, or preterm delivery). Maternal KIR gene content and HLA-C genotypes to allele level in couples experiencing recurrent miscarriage and controls.	The activation spectrum of KIR-HLA-C compound genotype for NK cells may contribute to the immunological etiology of recurrent miscarriage.
[47]	Activating KIRs	40 women with unexplained recurrent miscarriage and 90 controls. KIR genotyping.	Shifted balance of KIRs toward an activating state in NK cells may contribute to recurrent miscarriage.
[48]	Inhibitory KIRs	Retrospective study that included 291 women, with recurrent miscarriages or recurrent implantation failure, who had a total of 1304 assisted reproductive cycles. KIR genotyping.	These new insights could have an impact on the selection of single embryo transfer in patients with miscarriages or recurrent implantation failure, and with a KIR AA haplotype.
[49]	KIR2DS1/HLA-C2	The frequencies of KIR and HLA-C1 and HLA-C2 genes were evaluated in 139 women with ≥2 consecutive spontaneous pregnancy losses.	KIR and HLA-C genotyping is important for predicting immune-related problems in women with recurrent pregnancy loss women.

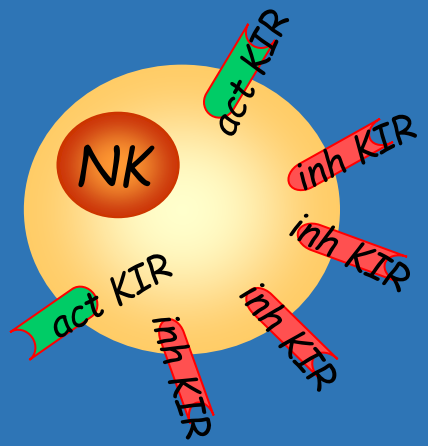
40. Varla-Leftherioti M, Spyropoulou-Vlachou M, Niokou D, Keramitsoglou T, Darlamitsou A, Tsekoura C, et al. Natural killer (NK) cell receptors' repertoire in couples with recurrent spontaneous abortions. Am J Reprod Immunol. 2003;49:183–91 Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12797525.
41. Witt CS, Goodridge J, Gerbase-DeLima MG, Daher S, Christiansen FT. Maternal KIR repertoire is not associated with recurrent spontaneous abortion. Hum Reprod. 2004;19:2653–7.
42. Flores AC, Marcos CY, Paladino N, Arruvito L, Williams F, Middleton D, et al. KIR receptors and HLA-C in the maintenance of pregnancy. Tissue Antigens. 2007;69(Suppl 1):112–3.
43. Wang S, Zhao YR, Jiao YL, Wang LC, Li JF, Cui B, et al. Increased activating killer immunoglobulin-like receptor genes and decreased specific HLA-C alleles in couples with recurrent spontaneous abortion. Biochem Biophys Res Commun. 2007;360:696–701.
44. Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. Hum Reprod. 2008;23:972–6.
45. Vargas RG, Bompeixe EP, França PP, Marques de Moraes M, da Graça Bicalho M. Activating killer cell immunoglobulin-like receptor genes' association with recurrent miscarriage. Am J Reprod Immunol. 2009;62:34–43 Available from: http://www.ncbi.nlm.nih.gov/pubmed/19527230.
46. Faridi RM, Agrawal S. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. Hum Reprod. 2011;26:491–7.
47. Ozturk OG, Sahin G, Karacor EDZ, Kucukgoz U. Evaluation of KIR genes in recurrent miscarriage. J Assist Reprod Genet. 2012;29:933–8.
48. Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, et al. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. Hum Reprod. 2014;29:2637–43.
49. Dambaeva SV, Lee DH, Sung N, Chen CY, Bao S, Gilman-Sachs A, et al. Recurrent pregnancy loss in women with killer cell immunoglobulin-like receptor KIR2DS1 is associated with an increased HLA-C2 allelic frequency. Am J Reprod Immunol. 2016;75:94–103.

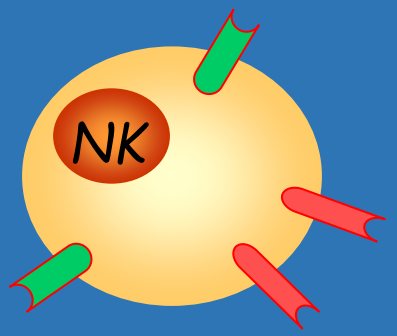
Díaz-Peña R Assist Reprod Genet. 2019 May;36(5):827-835

women with unexplained RM have a limited inhKIR repertoire

Varla-Leftherioti et al, Am J Reprod Immunol (2003)

fertile





RSA

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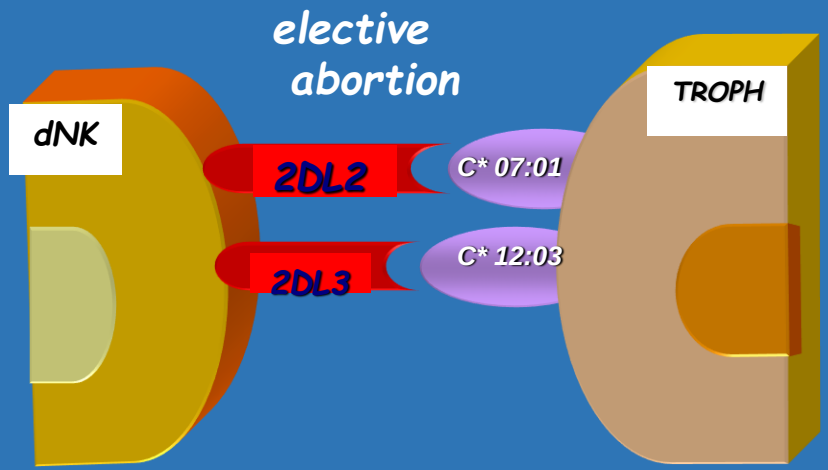
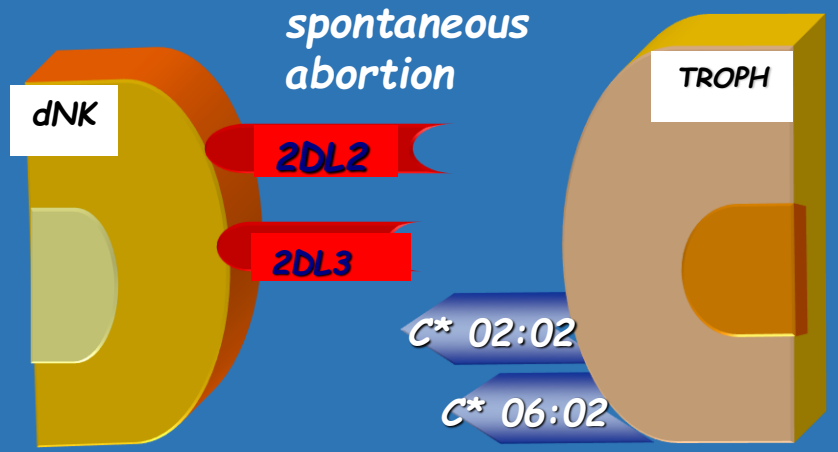
in some cases of spontaneous abortions, maternal inhKIRs do not find their specific HLA-C ligands on trophoblast (epitope mismatch)

Varla-Leftherioti et al, Hum Immunol 2005;66:65-71

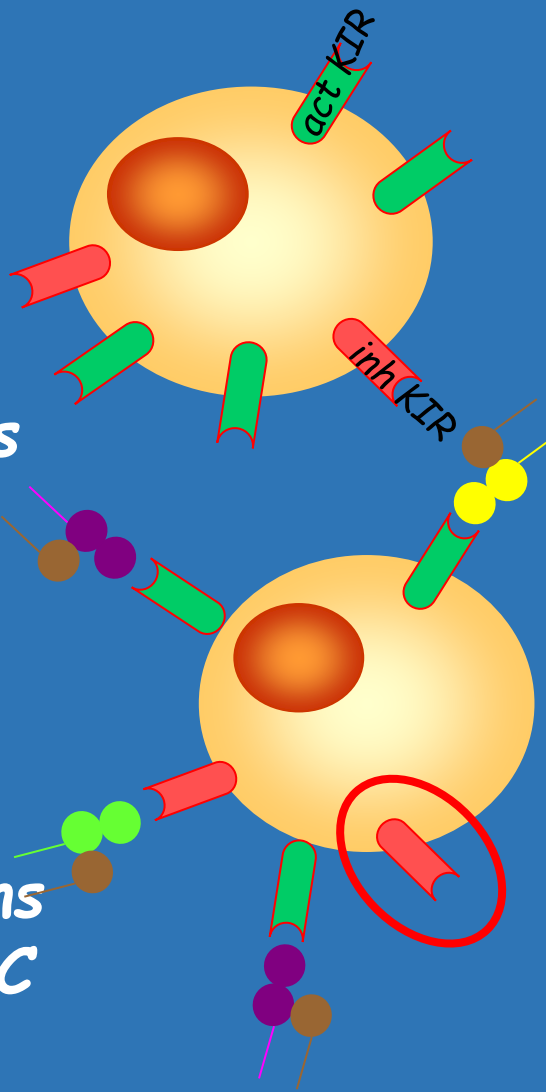




RM and RIF

inhKIR/actKIR

Aborters (allo)	1.9
RIF	1.9
Fertile	2.6



an imbalance in favour of activating KIRs

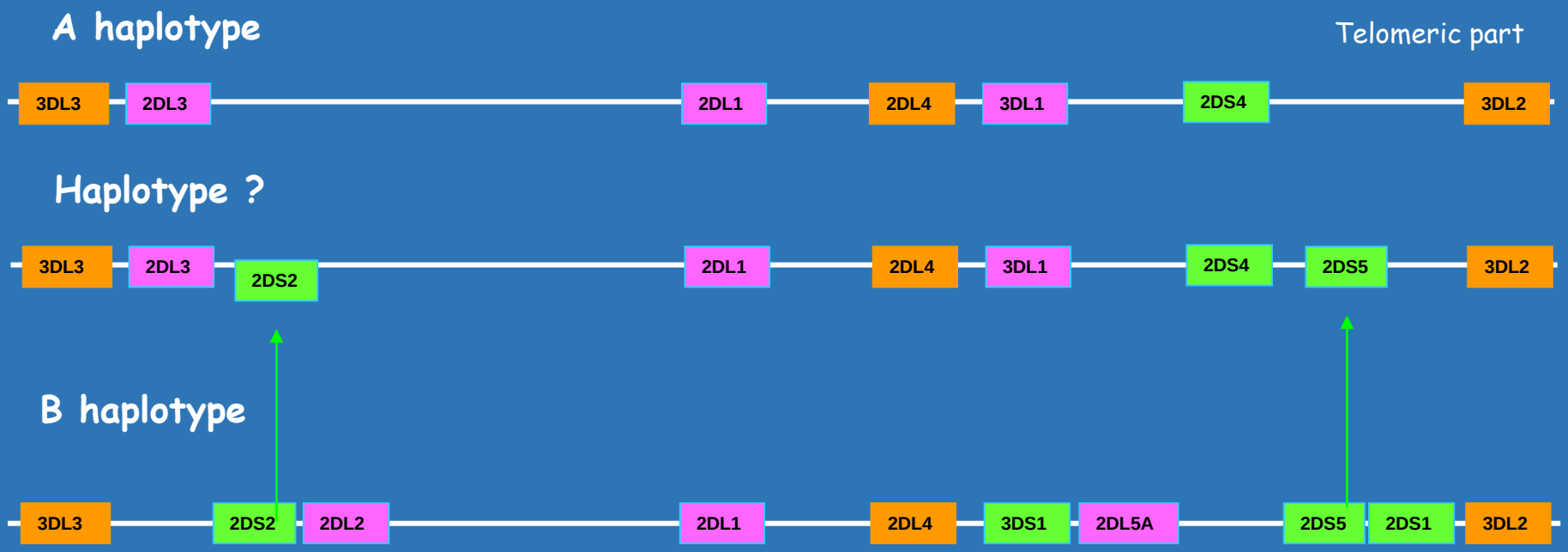
and/or

lack of specific inhKIR/HLA-C interactions in the presence of specific actKIR/HLA-C interactions

Varla-Leftherioti et al Tissue Antigens 2010

RM and RIF

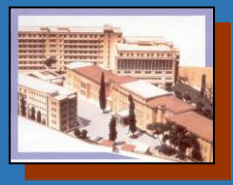
women with alloimmune RM possess the standard receptors of the KIR A haplotype combined with extra activating KIR/s of the haplotype B



Varla-Leftherioti et al Tissue Antigens (2007)

D6.17 Maternal KIR repertoire and KIR/HLA-C recognition model in early pregnancy and implantation failure **(Teams 1b, 21)**

All subjects were treated in Obstetrics and Gynecology Departments of "Helena Venizelou" Maternity Hospital and written informed consent to participate in the study was obtained. All study participants were Caucasian.

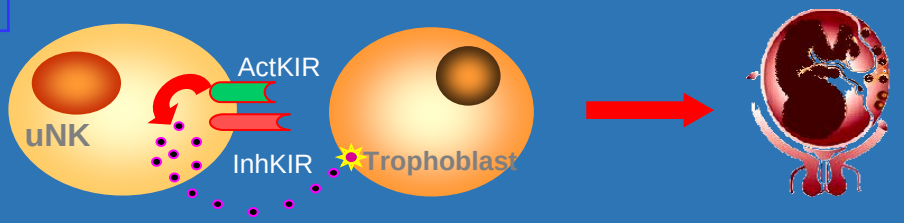


the distribution of inhKIR receptors and inhKIR/HLA-C combinations at the feto-maternal interface by direct genotyping of trophoblastic cells

cases of women who were undergoing vacuum uterine curettage for therapeutic termination of first trimester missed pregnancy or elective termination of normal pregnancy

	RM	Controls
Decreased KIR2DL1 (strong inhKIR)	+	-
Limited inhKIR repertoire	+	-
KIR2DL3-C1 Weak inhibition	+	-
KIR2DL1-C2 Strong inhibition	-	+
Embryo's C1C1	21,4%	**35,3%**
Embryo's C2C2	**23,8%**	27,5%

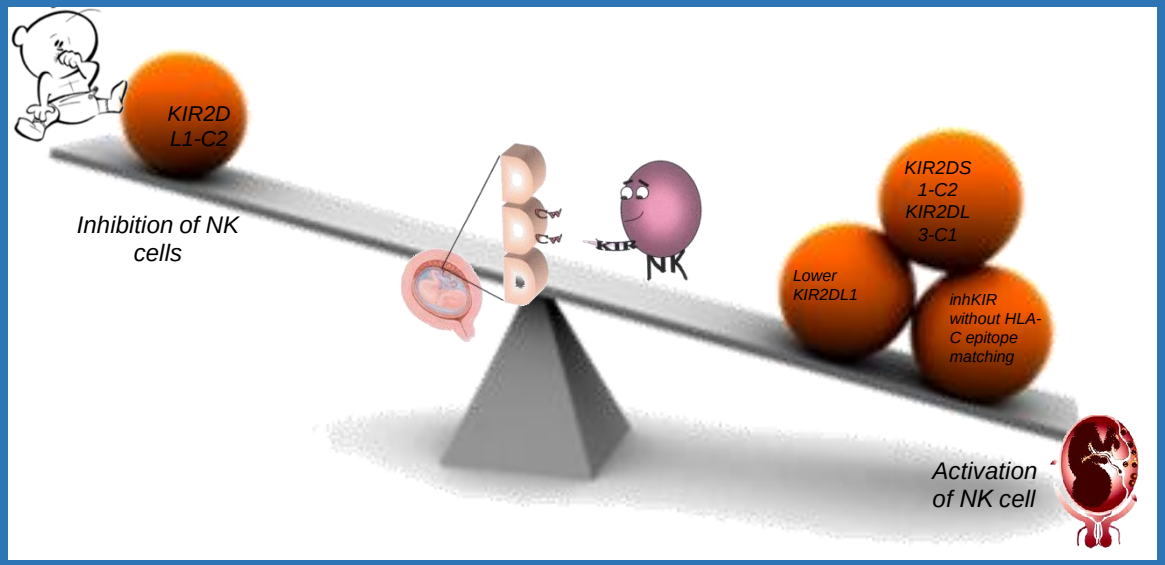
The results support the hypothesis that if trophoblastic HLA molecules are not recognized by dNK cell inhibitory receptors, the activation of NK cells is not inhibited and they attack trophoblast



women with unexplained RM or RIF

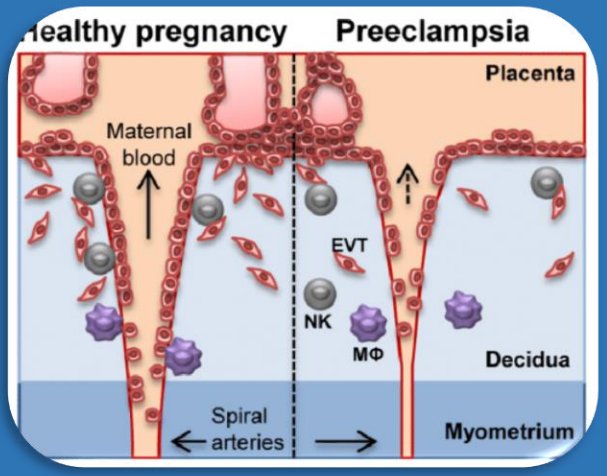
have a limited inhKIR repertoire

lack of maternal inhKIR/fetal HLA-C epitope matching



may give to aborters a higher potential for dNK cell activation, thus an increased risk for an adverse reproductive outcome

Preeclampsia



Decrease trophoblast invasion and size of spiral arteries

- In women with KIR *AA* haplotype there is an increased prevalence of preeclampsia
- Prevalence entirely due to pregnancies where the fetus genotype is either homozygous *C2* or heterozygous *C1C2*

Hiby et al, J Exp Med (2004)

- *KIR2DL1A*, not *KIR2DL1B*, associates with increased risk of preeclampsia

Huhn O. J Immunol. (2018)

Severe preeclampsia

no influence of HLA-C/KIR genetic variation

Larsen TG · Placenta (2019)

Can the detection of KIR/HLA-C combinations be applied in practice to diagnose the reason of abortion or preeclampsia?

The evaluation of KIR-HLA interactions is difficult

Same cell may co-express both activating and inhibitory KIRs

Different activating and inhibitory KIRs may have the same MHC ligand

Receptors of other families may also be expressed on the same cell

KIRs are expressed not only on NK but T subsets as well

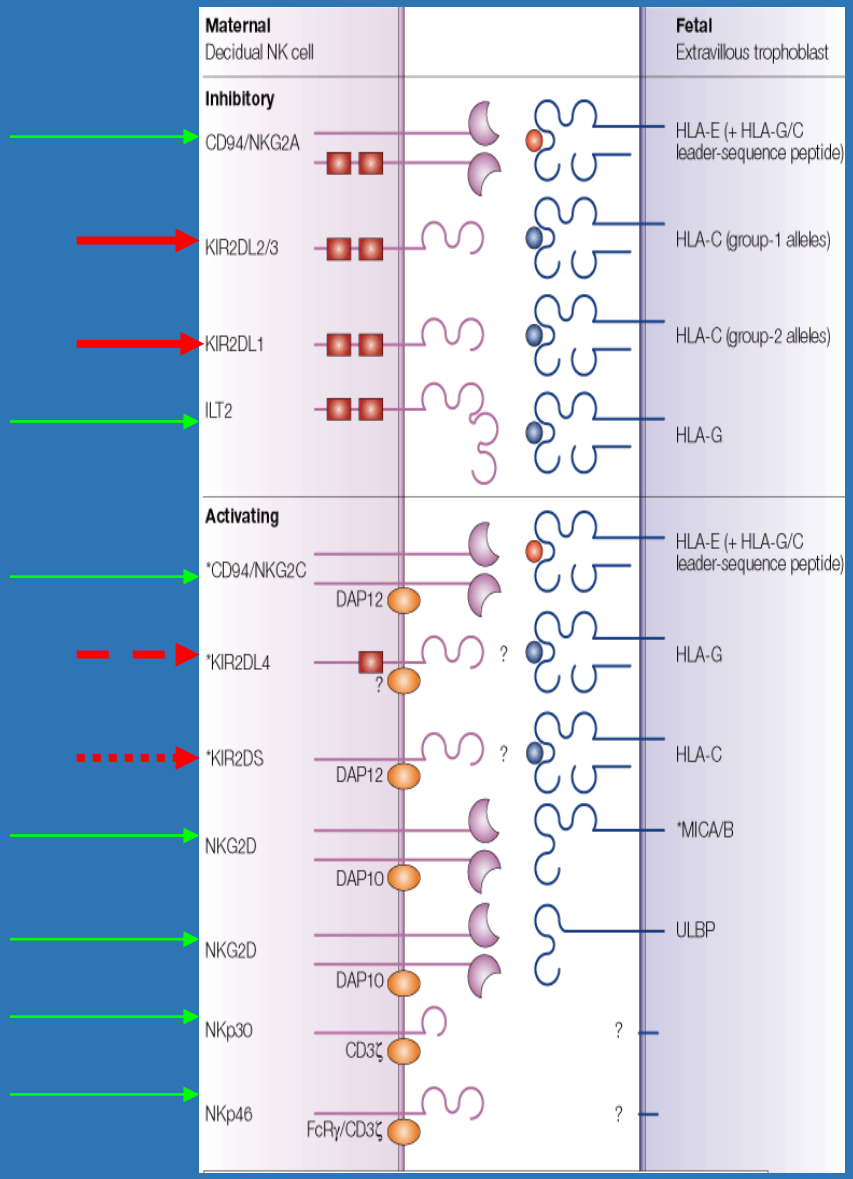
The ligands of most activating KIRs are unknown

Different KIRs bind their receptors with different strength

Particular inhKIR-C interactions provide different degree of inhibition

Particular actKIR-C alleles are associated with more responsive NKs

The final NK cell action is the result of cumulative interactions of different maternal ihn and act receptors with different self and non-self trophoblastic molecules



Recommendation

RM

RIF

Table 1 - A summary of all evidence-based diagnostic tests regarding the evaluation of RPL according to ESHRE (2017) and ASRM (2013) guidelines.

	ASRM guidelines	ESHRE guidelines
Infectious causes	Not recommended	Not recommended
Male Factors (Sperm DNA fragmentation)	Not recommended	Not recommended
Allo-Immune factors (HLA, anti-HY, cytokine and natural killer testing)	Not recommended	Not recommended
Health behavior modifications (Tobacco use, alcohol use, obesity)	History recommended	History recommended

Abbreviations: LA, lupus anticoagulant; aCL, anticardiolipin; aβ2GP1, anti-β2-glycoprotein 1; HLA, human leukocyte antigens; HSG, hysterosalpingogram; SHG, sonohysterogram; TPO-Ab, thyroid peroxidase antibodies; TSH, thyroid stimulating hormone; CGH, comparative genomic hybridization; PCOS, polycystic ovary syndrome.

Table 2. Summary of evidence for investigations into RIF.

Immunological disorders and thrombophilia	Uterine natural killer cells	Yello
	Peripheral natural killer cells	Red
	Endometrial cytokines	Red
	Genital microbiome	Red
	Peripheral blood cytokines	Red
	HLA incompatibility	Red
	Inherited thrombophilia	Red
	Antiphospholipid antibody syndrome	Yellow
Endocrine factors	Thyroid function test	Green
	Thyroid antibody testing	Yellow

	Is there evidence of an association between the test result and miscarriage?	Is there evidence that the association is contributory to miscarriage risk?	Is there evidence that the test result has prognostic value?	Is there evidence that treatment based on test results improves outcomes?
Immune testing (human leukocyte antigen compatibility, human leukocyte antigen class II, human leukocyte antigen-G, KIR and human leukocyte antigen-C, cytokines, and natural killer cells)	Little data	Little data	No data	No data
Anti-HY immunity	Moderate	Yes	Yes	No data
Antinuclear antibodies	Yes	Little data	Unclear	No data
Ho			No	Possibly (metformin treatment)
Vit			ittle data	No data
Sp			Unclear	No

Loss.[2] KIR=killer immunoglobulin-like receptor.

[53]

Recommendation (updated in 2022)

Human Leukocyte Antigen (HLA) determination in women with RPL is not recommended in clinical practice. Only HLA class II determination (HLA-DRB1*15:01, HLA-DRB1*07 and HLA-DQB1*05:01/05:02) could be considered in Scandinavian women with secondary RPL after the birth of a boy, for explanatory and prognostic purposes.

Conditional ⊕◯◯

Justification

	Association	Contributing factor	Prognosis	Treatment
HLA-compatibility	Controversial evidence	NA	No prognostic potential	NA
HLA class II: HLA-DR and HLA-DQ (maternal)	Strong, but only shown in Scandinavian women	YES, especially for secondary RPL after first born boy	Negative impact on future live birth	None available
HLA-G	Significant but weak	No data	No data	NA
KIR and HLA-C	Controversial evidence	No data	No data	NA

Update 2021

HLA determination in women with RM is not recommended

KIR or KIR/HLA-C typing is not suitable for diagnostic and therapeutic purposes at present

Use of KIR and HLA C genotyping

Selection of gametes from donor with specific genotypes

Improve the probability of a successful pregnancy

?

A strikingly lower miscarriage rate was reported in women with KIR-A with partners carrying HLA-C2, who were given either eggs from donors with unknown HLA-C status, or donors known to be HLA-C1C1

Alecsandru D, et al. Maternal killer-cell immunoglobulin-like receptor (KIR) and fetal HLA-C compatibility in ART-oocyte donor influences live birth rate. 2016

Another group given egg from donors known to be HLA-C2C2 (potentially detrimental) were given 'rescue' medical intervention with the immune 'activating' hormone G-CSF . And that strategy was also shown to produce higher live birth rates than those given random egg donors

Cruz M, et al. Use of granulocyte colonystimulating factor in ART treatment does not increase the risk of adverse perinatal outcomes. Reprod Biomed Online. 2019

Genetic testing for KIR/HLA-C implies that different immune therapies and strategies may be best for particular couples

If a woman is known to be KIR-A (with inhibitory NK receptors), immune suppressive therapy may be detrimental and contraindicated

If a woman is KIR-B (with activating receptors), empirical immune suppression might be worth trying.

Couples with KIRA/HLA-C2 may be more effectively treated with immune activators such as G-CSF, or even the endometrial scratch

Achilli C, et al. Fertil Steril. 2018

More studies are needed

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