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The Immunological Investigation of Recurrent Failed IVF and Recurrent Miscarriage

REPRODUCTIVE IMMUNOLOGY CENTRE
DEPT OF IMMUNOLOGY
ST HELIER HOSPITAL, CARSHALTON, SURREY

Recurrent Miscarriage (RM)

- Affects 2-4% of all couples
- Cause is unknown in many cases
- Definition varies between different studies/centres
- Preterm delivery, small for gestational age, perinatal loss and Caesarean section are all increased in women with RM whose pregnancies progress beyond 24 weeks

Recurrent Miscarriage (RM) – Causes

- Unexplained (10-15%)
- Chromosomal defects (C16,18,21,X) (?%)
- Infection (1%)
- Increased tendency to coagulation (10-13%)
- Endocrine/hormonal abnormalities (20%)
- Uterine/Cervical abnormalities (5-10%)
- Immune problems (30%)

Autoimmunity and RM (1)

- Anti-thyroid antibodies are seen in 20-40% of women with RM/RFI.
- Anti-nuclear abs even in low titre may be associated with RM/RFI.
- Anti-Ro abs associated with CHB but also more frequent in RM.
- Anti-phospholipid antibody syndrome (APAS). Most important autoimmune problem in RM - ?also important in early RFI.
- APAS associated with pregnancy loss in 55%

Autoimmunity and RM (2)

Anti-Phospholipids antibodies assoc with:

- Recurrent miscarriage
- Uteroplacental insufficiency and complications
- Pre-eclampsia (Toxaemia of pregnancy)
- Premature separation of the placenta (Abruption)
- Premature birth
- Thrombosis

Autoimmunity and RM (3)

APL abs impair implantation by:

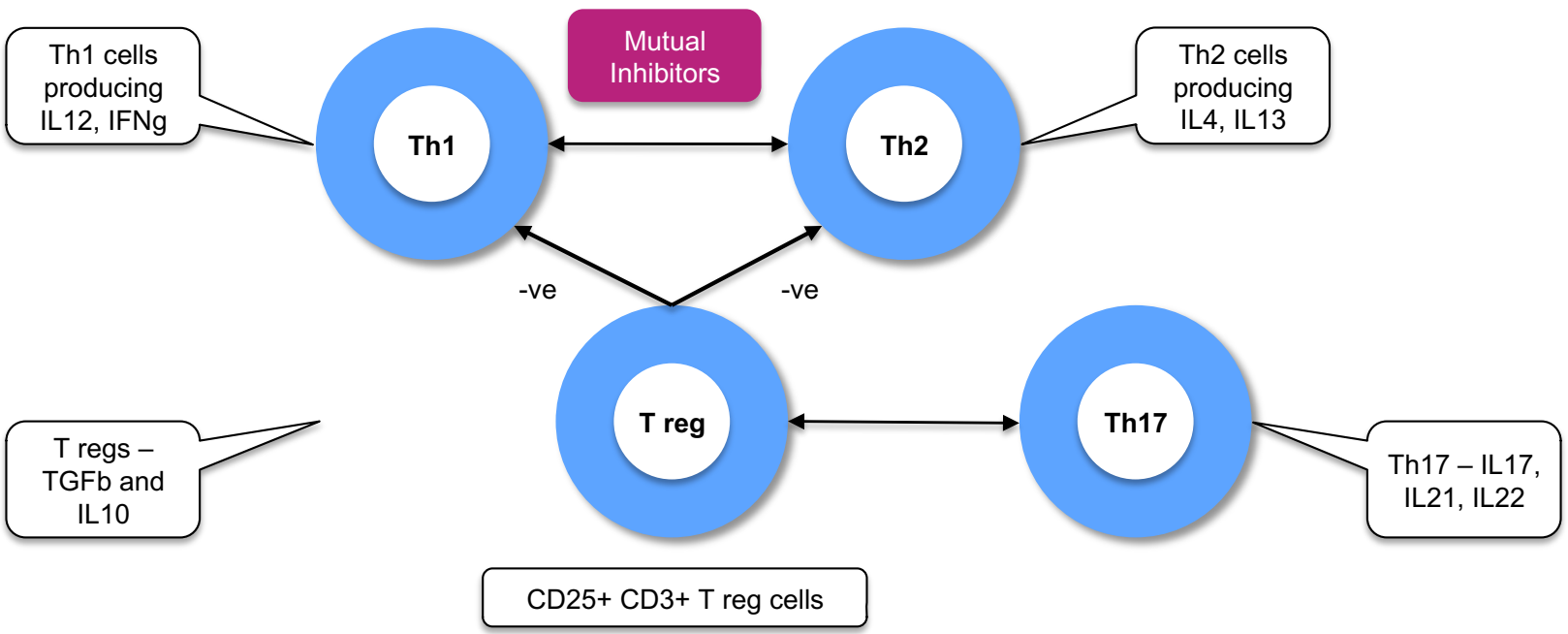
- Reducing HCG production by trophoblast
- Decreasing trophoblast invasion of decidua
- Reducing decidual function
- Impairing exchange function of placental cells
- Increasing death of placental cells

T cell interaction and the Concept of Th1/Th2 and Th17 and Tregs

CD4 T helper cells functionally divided:

- Th1 type cells produce IL2, IL12 & IFNg
- Th2 type cells produce IL4, IL5, (IL10) & IL13
- Th1 and Th2 type cells are mutual inhibitors
- T regulatory cells producing IL10 and TGFb regulate the activity of both Th1/Th2 cells.
- Th17 cell formation from naïve T cells is encouraged when IL6 predominates in the presence of TGFb.

Inter-relationship between the different T cell subsets



Pregnancy as a Th2 state and altered T cell function in RM

- First proposed by Wegmann et al (1993) using a mouse model and showing that foetoplacental cells secreted Th2 cytokines while body cells did not.
- Miscarriage may be due to inappropriate Th1 (Jenkins et al, 2000) and exaggerated Th17 type immunity (Nakashima et al, 2010; Wang et al, 2010) associated with insufficient Tregs function (Guerin et al, 2009).

How is a Th2 state initiated and maintained in pregnancy?

- Progesterone induced blocking factor –

  Produced by T gamma/delta cells, inhibits PLA2 and prostaglandin production leading to reduced NK cell activation and IL12 production. In high doses progesterone also upregulates HLA-G and increases LIF, M-CSF and Th2 cytokines.

- Placental suppressor factor
- Trophoblast cell-derived factor
- Early pregnancy factor (chaperonin 10)
- Cytokines such as IL10 and TGFb

Th17 and Tregs

- **Th17 cells:** Activate and recruit neutrophils.
- **Th17 cells**: Eliminate extracellular organisms particularly bacteria and fungi
- **Th17 cells:** Interleukin 17 produced by Th17 cells promotes inflammation
- **Tregs** regulate Th1, Th2 and possibly Th17 cells – appear critical to maintain pregnancy

Can external factors alter the normal state of T cell function in pregnancy?

- Viral infections – Increased Th1/Th17
- Endometrial bacterial and fungal infections – Th17 with reduced Tregs
- Systemic infection – Increased Th17
- Helminth infestation – Unclear ?Raised Th2
- Allergic disease - Unclear? Raised Th2
- Autoimmunity – Diminished Tregs? increased Th17

Autoimmunity, infection and the balance of T cell immunity

Bansal. RIC – UK. AJRI. 2010

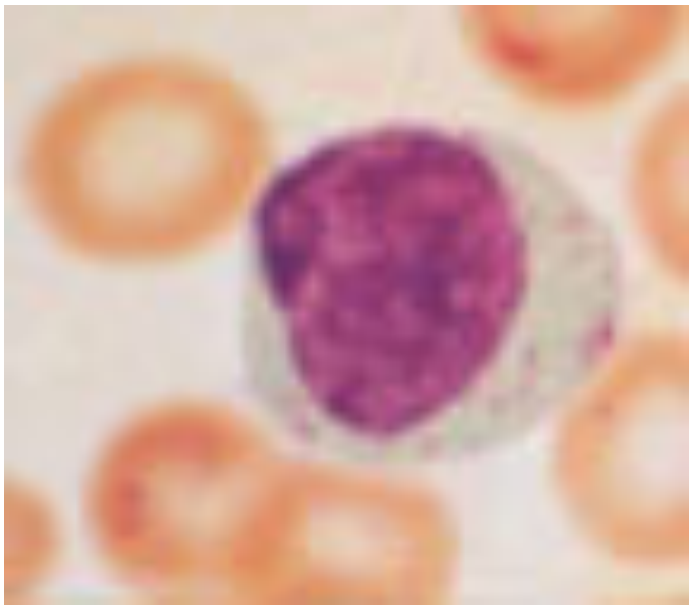
- Successful pregnancy requires a healthy balance of T and NK cell function.
- Overall, pregnancy is a Th2 predominant state where Tregulatory function inhibits excessive autoimmunity and Th1 function.
- The presence of autoimmunity (thyroid, ANA, APAS) suggests diminished Treg function which is associated with increased Th17 function and leads to increased NK cell activity.
- Infection is associated with raised TNFa and IL6 which leads to a relative increase in Th17 cells at the expense of Tregs.

NK cells in RM (1)

- NK cells may be divided into those that are like T cells in which case they are CD3-/CD8+ and those that are CD3-/CD8-.
- NK cells kill targets if they do not have correct expression of certain self proteins.
- The endometrium has one of the highest proportion of NK cells and the NK cell is the most abundant immune cell at the site of uterine implantation
- These NK cells are CD16- and CD56 bright.
- The precise function of these NK cell is complex.

NK cells in RM (2)

- In humans, NK cell numbers have been considered previously to correlate with poor outcome after IVF.
- RIC has shown NK cell activation to be the more important factor.
- NK cell activation is suggested by the expression of CD69 and HLA DR.
- CD69 is capable of inducing NK cell cytotoxicity, proliferation and cytokine release which can harm the developing embryo.



NK cytotoxicity and predicting pregnancy

- Matsubayashi et al, 2005 Am J Reprod Immunol
- Peripheral NK activity of 94 infertile women who despite treatment were unable to conceive for 6 or more months (mean; 2.4 years) and followed for 2 years.
- Peripheral NK activity measured by chromium-51 release cytotoxicity assay in 77 women who could be monitored.
- 28/77 who conceived had significantly lower peripheral NK activity (mean +/- S.D.; 34.5 +/- 13.8%) cf the 49/77 who had not conceived (42.3 +/- 13.3%) (P = 0.017).
- They suggest that elevated peripheral NK activity in patients with unexplained infertility is a risk factor for attaining pregnancy success.

The Value of enumerating activated NK cells RIC – *Hum Reprod. 2005; 20(5): 1272-6*

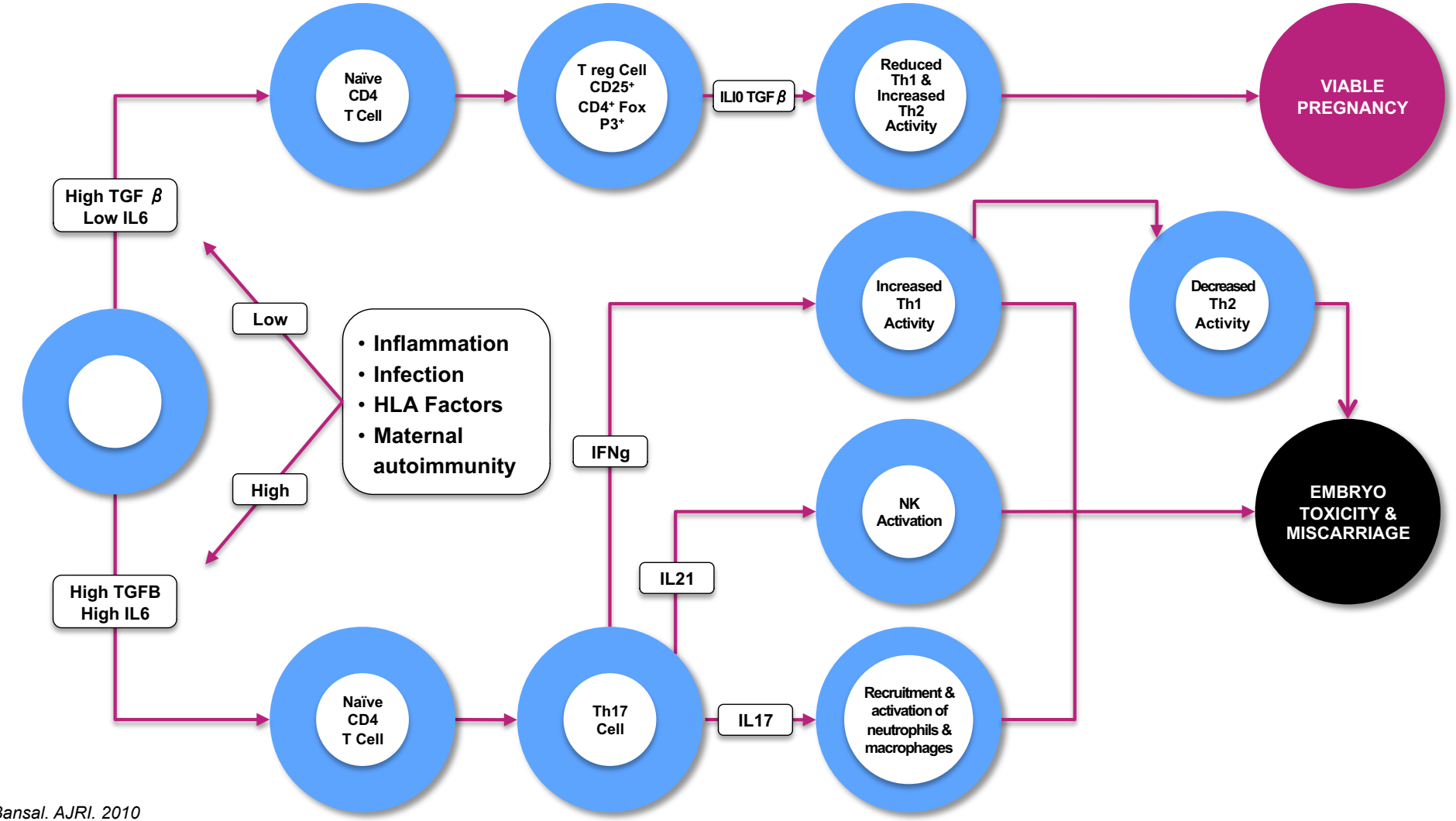
	CD69 NK cells <1	CD69 NK cells >1	P-value
Pregnancy rate	48.3%	23.1%	0.006
Live birth rate	40.2%	7.7%	P<0.0001
Miscarriage rate	16.7%	66.7%	0.005

How can RM/RFI be predicted?

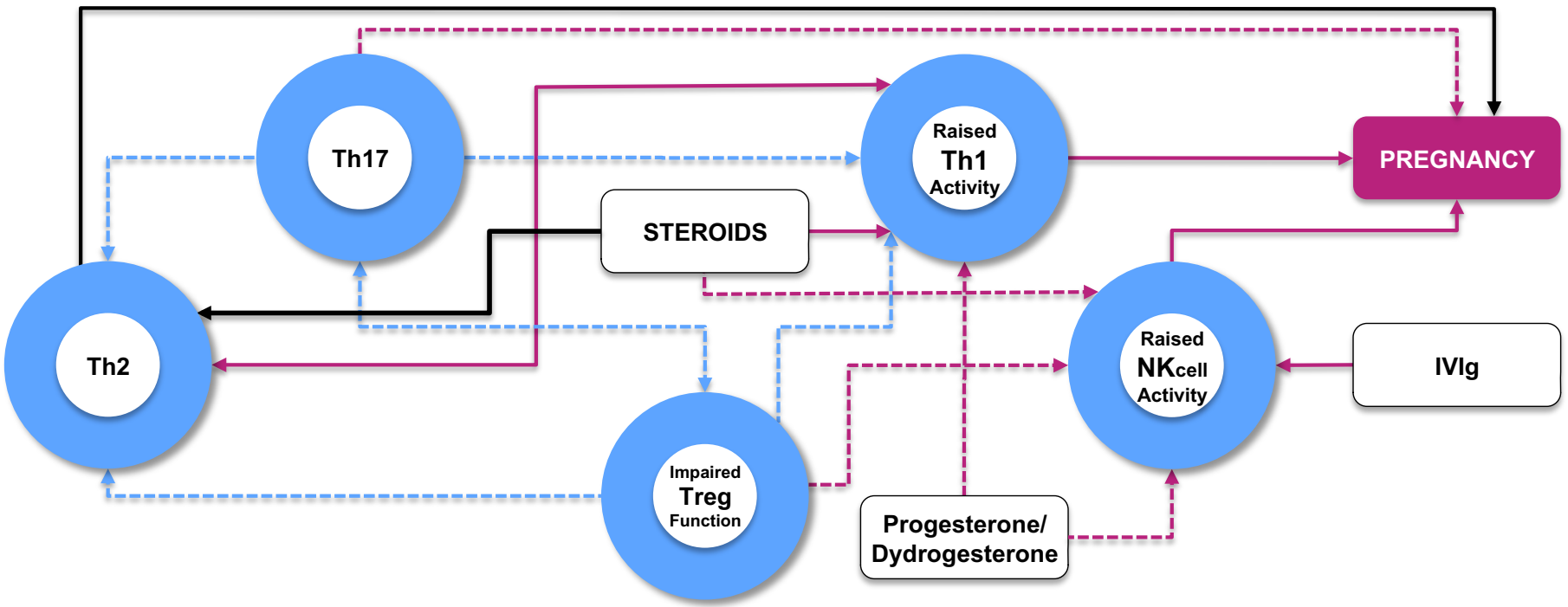
- Past obstetric history
- Auto-antibodies – ACl, LAC, ANA, TPO
- Procoagulant factors
- Endometrial pathogens
- Activated NK cell enumeration/NK cytotoxicity

Current therapies for RM/RFI

- Low dose aspirin – of little benefit by itself
- Low dose aspirin and heparin - beneficial
- Immunosupression
  (dampen an excessive Th1 response)
- Paternal leucocyte infusions
  (some evidence that paternal leucocytes selectively increase IL4 in one way MLC but most recent work shows it to be ineffective perhaps even harmful)
- Prednisolone
  (helpful in reducing inflammation and excessive T cell and NK cell activity)
- Intravenous immunoglobulin
  (mechanism unclear although thought to reduce Th1/ Th17 cytokines by T/NK cells, reduce NKCC by action on FcGR and block paternal derived foreign molecules)



Bansal. AJRI. 2010



— Suppression — Promotes — Interaction & Control

(Solid lines indicate definite evidence, while broken lines indicate some evidence)