Role of cytokines in endometrial-peritoneal crosstalk and development of endometriosis.



CK Kyama, MSc, TM D'Hooghe, MD, PhD,
Leuven (Belgium) and Nairobi (Kenya)
ESHRE-SGI Endometrium Symposium
Glasgow, UK, 17th March 2009

Leuven University Hospitals

Leuven University Fertility Center

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L Meeuwis
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C Tomassetti
S Pelckmans
P De Loecker
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I Thijs
Ph Albertyn
V. Vloeberghs

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CERTIFICATE OF ACCREDITATION

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This is to certify that the Quality Management System of

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ISO 9001 : 2000

The Quality Management System is applicable to:

Multidisciplinary and evidence based management of couples with fertility problems.

Approval Certificate No: 459041

Original Approval: 30 November 2004
Current Certificate: 30 November 2004
Certificate Expiry: 30 November 2007

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Leuven Endometriosis Research Group/Network: 8 PhD students

Clinical Leuven

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C Meuleman
L Meeuwis
K Peeraer
C Tomassetti
S Pelckmans
P De Loecker
V Vloeberghs

URO
B. VCleynenbreugel

GE surgery
A D'Hoore

Clinical Nairobi
D Chai

Postdocs Leuven
A Mihalyi; S. Debrock

PhD Students Leuven-Nairobi
C Kyama
A Atunga

PhD Students Leuven
A Vodolazkaia
A Fassbender
C Meuleman

PhD Students Leuven – int'ntl
P Simsa (Budapest)
A Bokor (Budapest)
H Falconer (Karolinska)

Research Nairobi
J Mwenda
D Chai
N Kulia
E Omolo
Veterinary staff
Animal attendants

Leuven Research coördinator
M Welckenhuysen

International collaborators
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G. Fried (Karolinska, Stockholm, SE)
G. Dunselman (Maastricht, NL)
A. Sharkey (Cambridge, UK)
F. Vilmos (Budapest, HUN)
K. Coleman (Oregon Primate Center, USA)
EU Network for Endometriosis (ENE)

INSTITUTE OF PRIMATE RESEARCH
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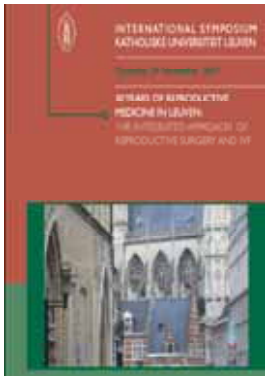
WHO COLLABORATING CENTRE



Disclosure

- Full Professor and Merck Serono Chair (2005-09) Reproductive Medicine (Leuven University)
- Clinical Head Leuven University Fertility Center
- Chair ESHRE Special Interest Group for Endometriosis
- PI ENDOCOST study

Disclosure

- Board member, WERF
- Editor-in Chief Gynecologic and Obstetric Investigation
- Research Associate and Chair International Advisory Board, Institute of Primate Research, Kenya
- Fundamental Clinical Investigator for endometriosis, Belgian Research Foundation

http://www.esnre.com

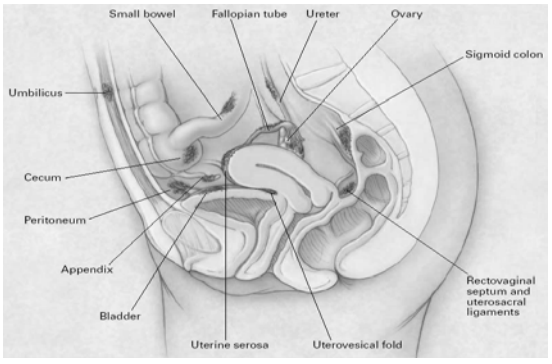
Endometriosis

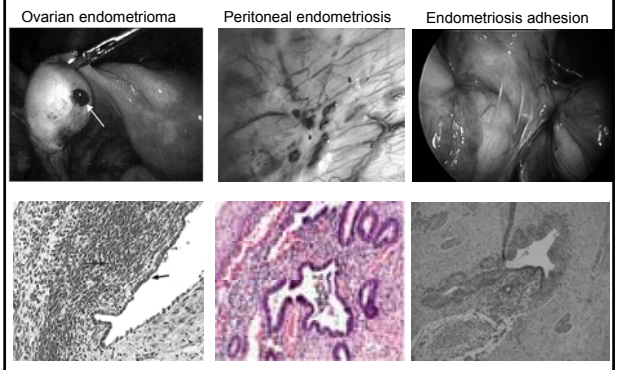
- **Defined as the presence of endometrial tissue (glands/stroma) outside the uterus**
- **Prevalence**
  - **7-15% of reproductive age women**
  - **up to 50% patients with pelvic pain/infertility**
- **Estrogen dependent**
  - **rare before menarche or after menopause**
- **Progressive**
  - **>50% women/baboons after 1-2 years**
- **Most common theory is "retrograde menstruation" (Sampson Hypothesis - 1927)**

Endometriosis EU14 million women

The presence of endometrium-like tissue outside the uterine cavity which induces a progressive, chronic, inflammatory reaction.

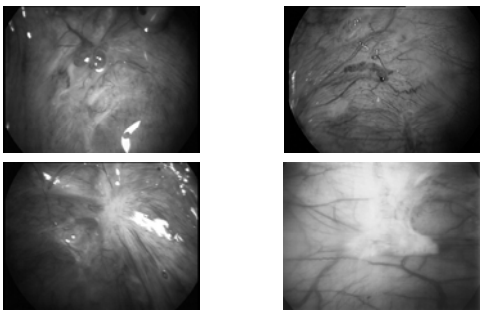
- Women of reproductive age
- All ethnic and social groups
- Pain, infertility, fatigue, …more?
- Diagnostic delay of 8.3 years



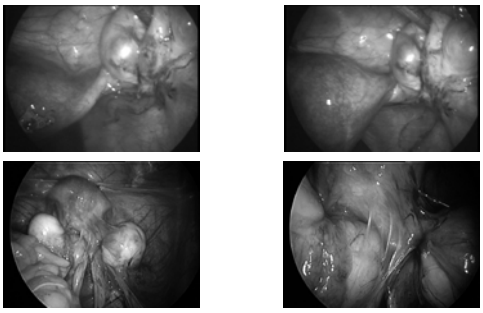


Clement., 2007; Hart., 2003; Prentice., 2001

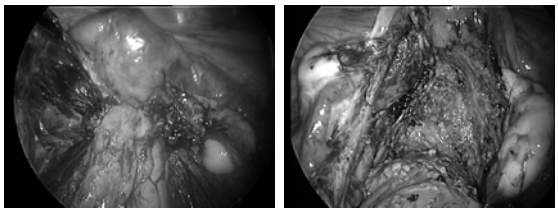
What does mild endometriosis look like?



What does severe endometriosis look like?



Excisional Laser Surgery for advanced endometriosis



The cost of endometriosis

DRUGS	DIAGNOSTICS	SURGERY	HEALTH CARE	OTHER
NSAIDs	Ultrasound scan	Laparoscopy	GP	ART
Progestagens	Internal scan	Laparotomy	Gynaecologist	A&E visits
c-OCP	MRI	Hysteroscopy	Nurse	Hospitalization
Danazol	Blood tests	Hysterectomy	Urologist	Alternatives
Gestrinone	Swabs	Endometrial ablation	Gastro-enterologist	Transportation
GnRH-a	Barium enema	Theater costs	Anaesthetist	Child care
Add-back HRT	Sigmoidoscopy		Radiologist	Work absence
Mirena coil	Endoscopy		Theatre staff	↓ productivity
Antibiotics	Bone scans		Haematologist	↓ education
Anti-depressants	X-rays		Counsellor	↓ activities
			Physiotherapist	
			Psychiatrist	

Comparative cost: endometriosis versus other chronic diseases

Endo-related cost estimates in USA 2002 (Simoens et al, 2007)

1. Annual (2002) healthcare costs + costs of productivity loss: $2,801 + $1,023 per patient = about $ 4000 per patient per year

2. USA cost per year for endo (2002)
$22 billion per year
(at 10% prevalence of endo among women of reproductive age)

3. **Endo cost considerably higher than cost related to Crohn's disease or to migraine in the USA for 2002 (Simoens et.al., 2007).**

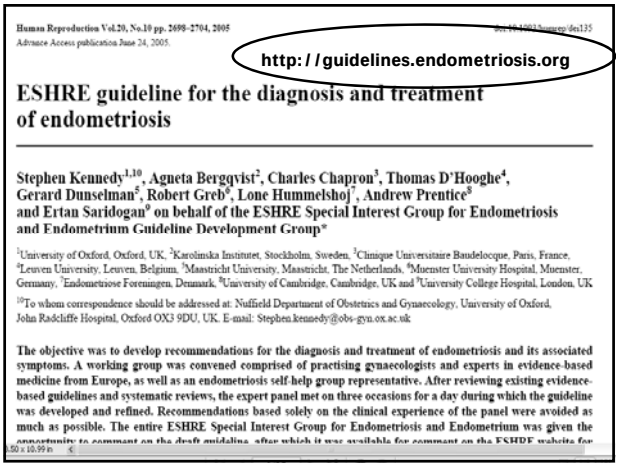
CALCULATION OF
ENDOMETRIOSIS COST IN EU
IS NEEDED FOR

INCREASED AWARENESS OF
ENDOMETRIOSIS IN

POLITICS DETERMINING
HEALTH POLICY
+ RESEARCH FUNDING

Role of ESHRE Special Interest Group for Endometriosis (SIGEE)

- Education and training
- ESHRE Guidelines for endometriois: Annual update via Working Group
- ESHRE endometriosis cost working group: ENDOCOST study



Human Reproduction Vol.20, No.10 pp. 2698–2704, 2005
Advance Access publication June 24, 2005

http://guidelines.endometriosis.org

ESHRE guideline for the diagnosis and treatment of endometriosis

Stephen Kennedy[1,10], Agneta Bergqvist[2], Charles Chapron[3], Thomas D'Hooghe[4], Gerard Dunselman[5], Robert Greb[6], Lone Hummelshoj[7], Andrew Prentice[8] and Ertan Saridogan[9] on behalf of the ESHRE Special Interest Group for Endometriosis and Endometrium Guideline Development Group*

[1]University of Oxford, Oxford, UK, [2]Karolinska Institutet, Stockholm, Sweden, [3]Clinique Universitaire Baudelocque, Paris, France, [4]Leuven University, Leuven, Belgium, [5]Maastricht University, Maastricht, The Netherlands, [6]Muenster University Hospital, Muenster, Germany, [7]Endometrioseforeningen, Denmark, [8]University of Cambridge, Cambridge, UK and [9]University College Hospital, London, UK

[10]To whom correspondence should be addressed at: Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK. E-mail: Stephen.kennedy@obs-gyn.ox.ac.uk

The objective was to develop recommendations for the diagnosis and treatment of endometriosis and its associated symptoms. A working group was convened comprised of practising gynaecologists and experts in evidence-based medicine from Europe, as well as an endometriosis self-help group representative. After reviewing existing evidence-based guidelines and systematic reviews, the expert panel met on three occasions for a day during which the guideline was developed and refined. Recommendations based solely on the clinical experience of the panel were avoided as much as possible. The entire ESHRE Special Interest Group for Endometriosis and Endometrium was given the

ESHRE Endometriosis Cost Working Group

- Initiative for ENDOCOST study
- 8 countries, 10 centers:
  Germany, Hungary, UK, Italy, Denmark, France, Netherlands, Belgium, Switzerland, USA (2)
- Retrospective/Prospective study (2009)
- Team per center: 1 gynecologist + 1 health economist
- Travel/lodging supported by ESHRE
- Collaboration with ASRM SIG Endometriosis
- Sponsored by World Endometriosis Research Foundation

European Network on Endometriosis

First ever EU research grant for endometriosis

1. Pan European epidemiological study
2. Internet based endometriosis gateway
3. Consolidate and formalise the European Alliance

- 8 Associate partners and 4 Collaborating partners
  - Endometriosis UK lead partner
  - Belgium, Denmark, Italy, UK
- Application scored very highly – 87/100 and received funding 300.000 Euro (2007-9)

Review

- Pathogenesis theories
- Baboon model for endometriosis
- PF Endometrial cells
- Endometrial-peritoneal adhesion
- Peritoneal inflammation
- Immuno tolerance/Auto-immunity

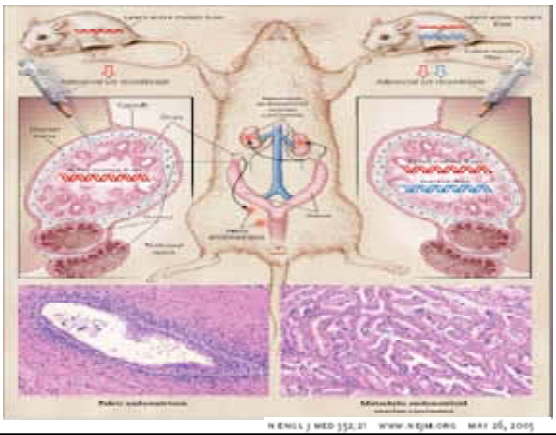
Pathogenesis of Endometriosis

Principal theories

- Retrograde menstruation (Sampson, 1927)
- Metaplasia theory (Iwanoff., 1898, Meyer., 1903)
- Induction theory (Levander and Normann., 1955)

- Immunological dysfunctions (Matarese et al., 2003)
- Environmental influences (Rier and Foster., 2002)
- Genetic predisposition (Montgomery *et al.,* 2008)
- Lymphatic or vascular distribution (Halban., 1924)
- Embryonic rests theory (Von Recklinghausen., 1896, Russell., 1899)

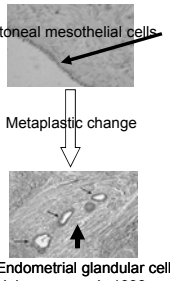
Mouse model



Pathogenesis of Endometriosis

- Ultrastructural studies

Metaplasia theory
(Iwanoff, 1898)



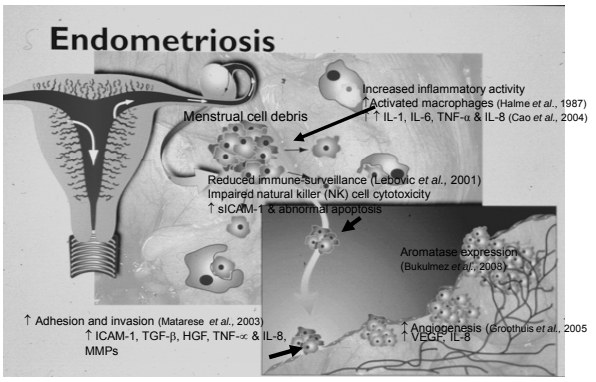
Endometrial glandular cells
Nakamura *et al*., 1993

In vitro study evidence that endometriotic lesions can arise by process of metaplasia from ovarian surface epitheluim (Matsuura *et al*., 1999)

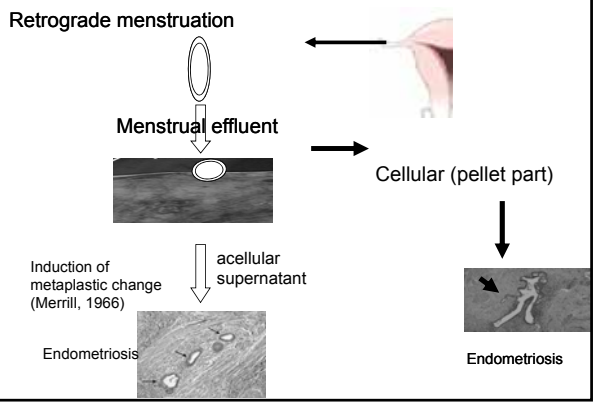
Peritoneal morphology

- In vitro evidence that endometriotic lesions can arise by metaplasia from ovarian surface epitheluim (Matsuura *et al*., 1999)
- Surface mesothelium of pelvic peritoneum adjacent to active endometriosis undergo a sequence of reactive change from flat cell to cuboidal or columnar epithelium (Ishimaru et al., 2004,Sugiwara et al., 1997)

Retrograde menstruation



Pathogenesis of Endometriosis



Active role of normal pelvic peritoneum

- Increased accumulation of macrophages and higher expression of HGF in adjacent cuboidal or columnar cells of pelvic endometriosis (Khan et al., 2004)

HGF may be involved in the transformation of peritoneal mesothelium ?
(Sugiwara et al., 1997, Khan et al., 2004)

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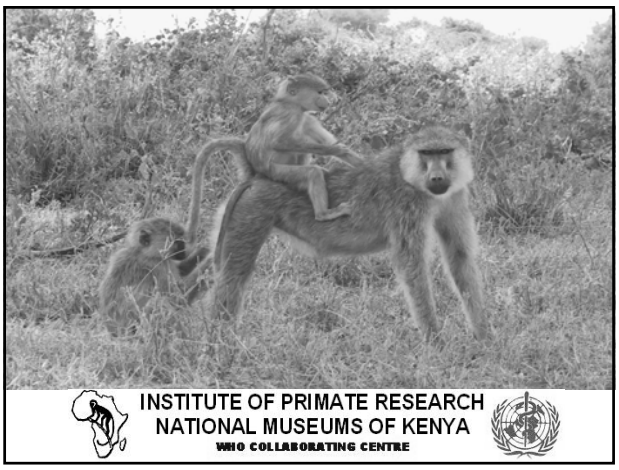
- Tolerance/Auto-immunity



***Institute of Primate Research* Nairobi, Kenya**

WHO Collaborating Center

Research areas:
Reproduction
Infectious Diseases
Ecology and Conservation

About 20 yrs research collaboration Leuven-Nairobi

- **1990-1993 Baboon model for Endometriosis, Institute Primate Research, Nairobi, Kenya**
- **1993-1995 Fellowship Reproductive Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, (JA Hill/ DJ Anderson) Endometriosis in baboons and women**
- **PhD Leuven 1994 Baboon as model for endometriosis**

20 yrs research collaboration Leuven-Nairobi

1998-2008: 50% fundamental clinical investigator (Flemish fund scientific research)

Clinical Leuven: Biobank frozen and paraffin mixed tissue, plasma, Peritoneal fluid and DNA + clinical database since 1998 (1863 patients)

Preclinical IPR Nairobi:

Baboon model: pathogenesis and testing of new drugs (prevention/treatment of endometriosis)

IPR International Advisory Board

- **Established 2007 – 3 meetings so far**
- **Initiative by NMK/IPR + supported by WHO (P. Van Look)**
- **Aim:**
- **advise Kenyan leaders about long term development of IPR into African Center of Excellence**
- **increase international research collaboration**

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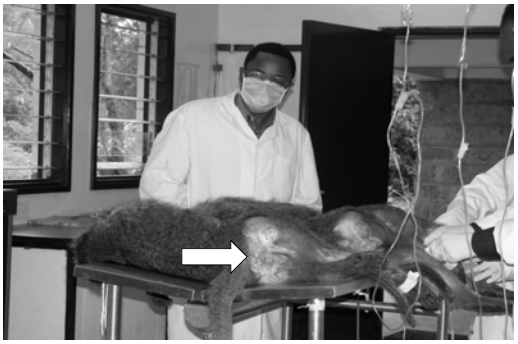
WHO COLLABORATING CENTRE

Institute of Primate Research, Nairobi, Kenya
WHO Collaborating Center

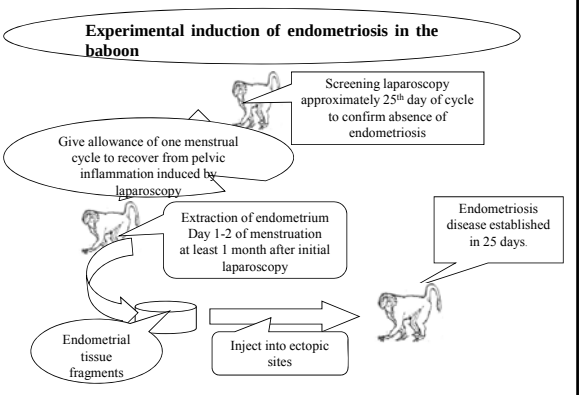


Advantages BABOON MODEL ENDOMETRIOSIS (1)

- **Not an endangered species/threat to agriculture in Africa**
- **noninvasive perineal skin monitoring for staging menstrual cycle,**
- **continuous breeder,**
- **larger and stronger primate than rhesus or cynomolgus monkeys, allowing repetitive blood sampling and complex experimental surgery**
- **spontaneous presence of peritoneal fluid**
- **Accessibility of the uterine cavity via the cervix (EM biopsy, embryo flushing, hysteroscopy)**

ADVANTAGES BABOON MODEL ENDOMETRIOSIS (2)

- **Spontaneous endometriosis: both minimal and disseminated/life threatening**
- **Adapted rAFS staging for endometriosis**
- **Induced endometriosis: more advanced stages of endometriosis after intrapelvic seeding of menstrual endometrium inside the pelvic cavity (D'Hooghe et al, 1995).**
- **In vivo culture model for study of early endometrial-peritoneal interaction.**



NEED FOR BABOON MODEL (3)

- **Validated for infertility**
- **Being validated for pain**
- **Prevention studies (prevent endometrial-peritoneal attachment)**
- **Treatment studies (reduce extent of induced endometriosis)**
- **Treatment of endometriosis-associated subfertility with standardization for:**

degree of endometriosis (amount EM for Ipseeding), ovulation (perineal cycle),

male factors (timed intercourse fertile male baboon, behavioral observation and PCT)

Ethics of endometriosis research in baboons at IPR

1. **Baboons are not an endangered species but represent a threat to agriculture in Africa**
2. **Baboons live in their natural habitat at IPR**
3. **Lack of other clinically relevant preclinical animal models to study cause-effect relationships: Only NHPs do have spontaneous/induced endo similar to the disease in women**
4. **Ethical need to show safety + efficiency of new drugs before application in women**

Ethics of endometriosis research in baboons at IPR

5. **For each project: double approval by ethical committees from both IPR and from Leuven University**
6. **Global level: capacity building of Primate Research Center in poor resource country could/should be seen as relevant effort in the context of North-South collaboration**

VALIDATION OF BABOON ENDOMETRIOSIS MODEL

- **Pub Med (updated 28th Jan 2009):**
- **Baboon AND Endometriosis N=62**
  - **34 Leuven-IPR Nairobi group (T. D'Hooghe)**
  - **14 Chicago group (A. Fazleabas)**
  - **6 San Antonio Group (B. Barrier)**
  - **8 others**

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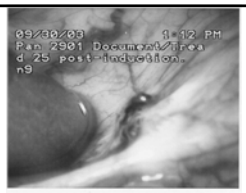
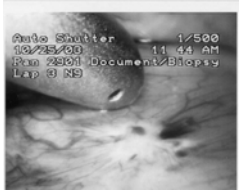
Endometrial changes in endometriosis: cause or consequence?

- Fazleabas et al, 2004

Baboons with induced endometriosis:

- Aromatase expression in endo lesions only after 10 months
- Downregulation of uterine receptivity markers as early as 1 to 4 months after induction





Review

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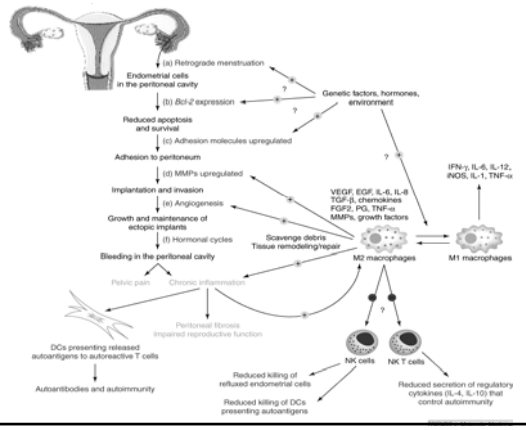
- Immune tolerance/ Auto-immunity

Matarese et al, Trends Molec Medicine 2003



PF endometrial cells and endometriosis: does retrograde menstruation exist?

- **PREVALENCE OF PF EM CELLS**
- **During menses (Reti et al, 1983): 24% (50% DII-III)**
- **During other phases of the cycle, most studies: 0-19% (23-67% after hysteroscopy or uterotubal flushing)**

- **PROBLEMS WITH STUDY DESIGN:**
- **? Cycle phase**
- **? Adequate PF cell preparation (cytospin vs cytoblock)**
- **? Adequate definition of morphology**
- **? Adequate immunohistochemical markers identifying EM epithelial, EM stromal, mesothelial cells and WBCs**
- **Endometrial-peritoneal adhesions occurs within 24 hours (Witz et al, 2000)**

QUANTITY OF PF EM CELLS -EXP. DATA(1)

Experimental in vivo data: positive correlation between weight of EM tissue used for intrapelvic seeding and extent of endometriosis in baboons (D'Hooghe et al, 1995)

Experimental in vitro data:EM fragments with intact microstructure express several adhesion molecules an adhere better to amniotic epithelium(van der Linden et al, 1995) and invade ECM earlier (Wild et al, 1994) than isolated or single EM cells.

QUANTITY OF PF EM CELLS-EPIDEMIOL (2)

Epidemiology: increased risk for endometriosis if

- short cycle length (Cramer et al, 1986; Arumugam and Lin, 1997) or longer menstrual flow (Cramer et al, 1986; Vercellini et al, 1997)
- if obstructed menstrual outflow: endometriosis in 66% (Olive and Henderson, 1987) or 77% (Pinsonneault and Goldstein, 1985) of women and in 3/3 baboons (D'Hooghe et al, 1994)

QUANTITY OF PF EM CELLS (3): CUMULATIVE RETROGRADE MENSTRUATION

- **BABOON MODEL FOR ENDOMETRIOSIS**
- Increased duration of captivity --> increased prevalence of endometriosis (D'Hooghe et al, 1996a)
- Spontaneous endometriosis is a progressive disease when followed by laparoscopies every 6 months during 2 years (D'Hooghe et al, 1996b)
- Baboons with an initially normal pelvis develop in 64% histologically proven minimal endometriosis after 32 months as assessed by laparoscopies every 6 months (D'Hooghe et al, 1996c)

Review

- Pathogenesis theories
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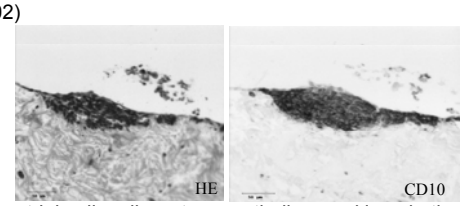
- Peritoneal inflammation
- Immunological tolerance
- Auto-immunity (Ab-mediated disease?)

Endometrial-peritoneal interaction

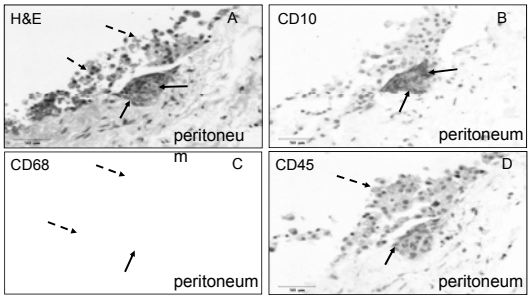
- Endometrium obtained during menstrual, follicular and luteal phase can adhere to autologous peritoneum in vitro (Debrock et al, 2002)



❖ endometrial cells adhere to mesothelium and invade the extracellular matrix (Witz *et al* 1999, Witz *et al*., 2001)

Pelvic inflammation and endometriosis

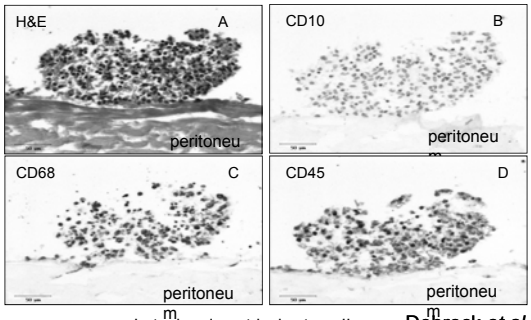
Peritoneal fluid pellet-peritoneal adhesion



Luteal endometriosis stage IV Debrock *et al*

Pelvic inflammation and endometriosis

Peritoneal fluid pellet-peritoneal adhesion



Luteal endometriosis stage II Debrock *et al*

Early Development of endometriosis in baboons

Development of endometriotic lesions after intrapelvic injection of menstrual endometrium is characterised

by a specific time-dependent histological process

Specific objectives

- To compare surface area of macroscopic lesions after day 1,3,6,9 and 15 post-induction
- To compare number of macroscopic endometriotic lesions after day 1,3,6,9 and 15 post-induction
- To confirm macroscopic endometriosis with histology to assess early endometrial-peritoneal interaction

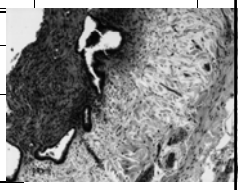
EXPERIMENTAL DESIGN

- Endometriosis was induced in 19 baboons with normal pelvis, as previously described [D'Hooghe, *et al*, 1995].
- A videolaparascopy was performed after 1 day (n=4),3 days (n=5),6 days (n=3),9 days (n=4), 15days (n=3) to document the number, surface and volume of endometriotic lesions and adhesions according to the rAFS [ASRM, 1996].

RESULTS

	Day 1	Day 3	Day 6
No of lesions (median, range)	4.5, 2-8 total: 19	8, 3-11 total: 38	7, 6-7 total: 20
Surface area lesion (mm^2)(median range)	46.5, 0-585 total: 678	344.5, 38-477 total:1413	93, 58-530 total: 678

	Day 9	Day 15
No of lesions (median, range)	8.5, 6-16 total: 39	16, 9-17 total: 42
Surface area lesion (mm^2)(median range)	88.13, 22-238 total: 436.3	67, 37.5-83 total:187.5



Classical endometriosis was observed day 3 (2/10 biopsies), day 6 (4/13 biopsies) and day 9(4/18 biopsies) post induction

Acellular menstrual fluid can induce endometriosis in baboons

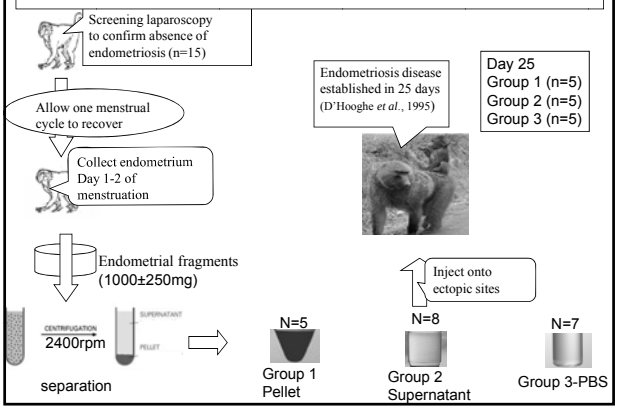
HYPOTHESIS

Endometriosis can be induced after IP injection of acellular menstrual endometrial fluid (supernatant)

Specific objectives

1. To compare surface area of macroscopic lesions between endometrial pellet, endometrial supernatant and PBS 25 days post induction
2. To compare number of macroscopic endometriotic lesions between groups
3. To confirm endometriosis with histological analysis and assess CD10 expression

EXPERIMENTAL DESIGN



RESULTS

rAFS endo stage (D'Hooghe *et al*., 1995).

Group 1 (Pellet) – stage I (n=1), stage II (n=3), stage III (n=1)

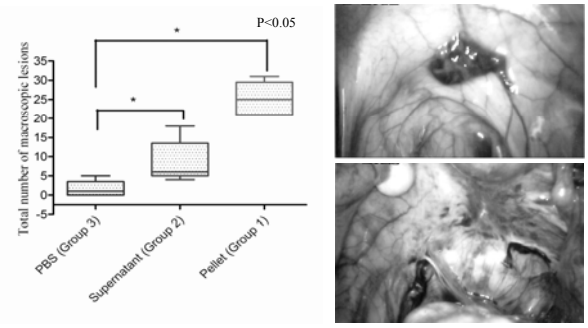
Group 2 (Supernatant) - stage I (n=5)

Group 3 (PBS) - stage 0 (n=3), stage I (n=2),

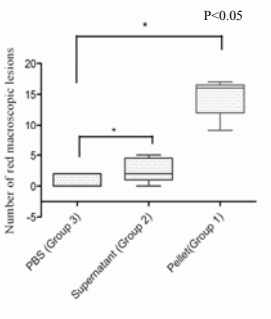
Dense adhesions noted in group 1(n=5)

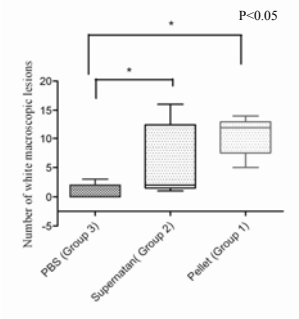
	Group 1	Group 2	Group 3
No of lesions (median, range)	25, 21 – 31 total: 126	6, 4 - 18 total: 43	1, 0 – 5 total: 8 *P* =0.027
No of lesion (red, white)	73, 53,	13, 30	4, 4
Surface area lesion (mm^2)(median range)	2, 0.25-250, total: 757	0.25, 0.25-25 total:64.25	1, 0.25 – 2 total: 7 *P* <0.0001
Surface area lesion (mm^2(red, white)	696.5, 60.5	37.25, 27	4, 3

Comparison of macroscopic endometriotic lesions

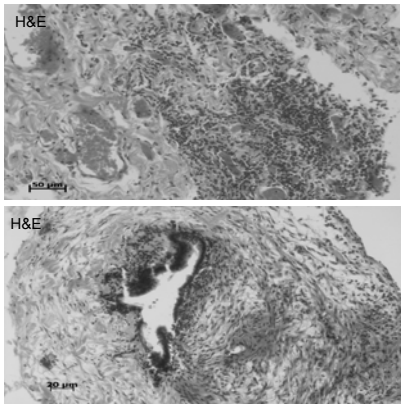


Comparison of endometriotic lesions phenotype

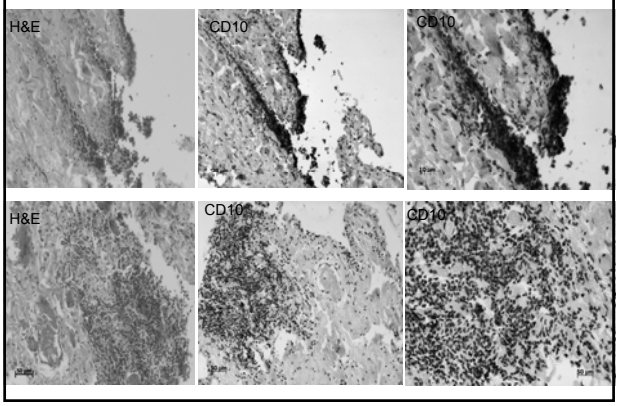




Presentation of endometriosis



Assessment of Stromal Cells



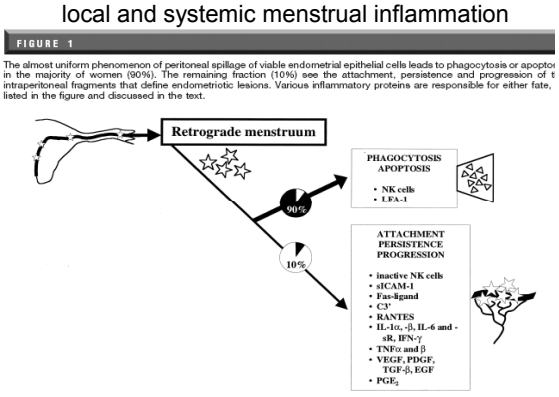
SUMMARY HISTOLOGY

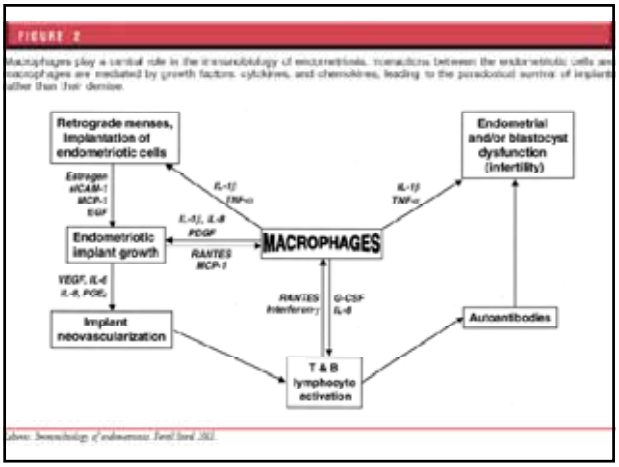
- ↑ number of lesions &surface area with a phenotype compatible with endometriosis in Group 1 than in Group 2 or in Group 3
- ↑ Classical endometriosis in Group 1 (8/12) than in biopsies from Group 2(1/14) or from Group 3 (0/6)
- ↑ **Stromal endometriosis** (Clement, 2007) was only observed in Group 1 (1/12 biopsies), **in Group 2 (4/14 biopsies),** and in no biopsies from Group 3
- CD10 immunohistohemistry staining confirmed presence of stromal cells

Review

- Pathogenesis theories
- Baboon model for endometriosis
- PF Endometrial cells
- Endometrial-peritoneal adhesion
- Endometrial/Peritoneal inflammation
- Immunological tolerance
- Auto-immunity (Ab-mediated disease?)

Why do not all women develop endometriosis: local and systemic menstrual inflammation





Endometriosis = *PF Inflammation*

- **Patients have chronic pelvic inflammation**
  - **↑ PF volume and PF WBC concentration**
  - **↑ activation of PF macrophages**
  - **↑ PF inflammatory cytokines/growth factors**
- **↑ pelvic inflammation in baboons after intrapelvic injection of endometrium (D'Hooghe et al, 2001)**
- **Pelvic inflammation (WBC X 3 increased) during menses compared to nonmenstrual phases in women (Debrock et al, 2000) and baboons (D'Hooghe et al, 2001)**

Menstrual > Luteal:
only significant differences in women with Endometriosis (Kyama et al, 2006 and 2007)

- **Endometrium:**
  higher expression of MMP-3, TNF-∝ and IL-8 menstrual phase > luteal phase
- **Peritoneum:**
  increased expression of RANTES, MMP-3, TGFbeta , ICAM-1, VCAM-1 and IL-6 in menstrual phase > luteal phase.

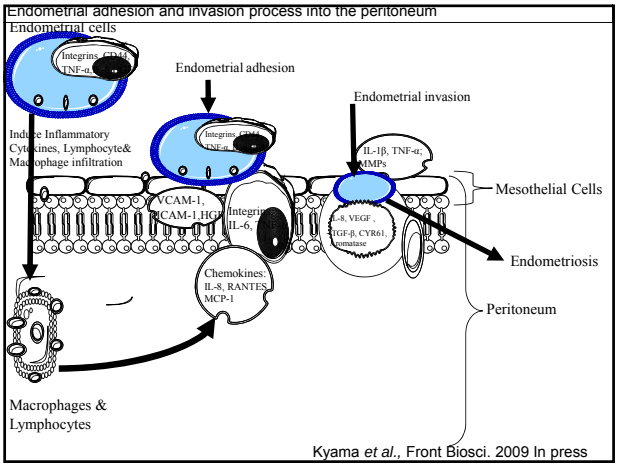
Endo = EM inflammation

Kyama et al, 2006 and 2007

- **Menstrual phase:**
  increased expression of TNF-∝, IL-8 and MMP-3, αV integrin, combined αVβ3 integrins mRNA levels in women with endo > controls
- **Luteal phase:**
  increased expression of IL-1β and RANTES in women with endo > controls
- **Menstrual or luteal phase combined:**
  higher aromatase expression in women with endo > controls

Endo = inflammation of nl peritoneum
(Kyama et al, 2006 and 2007)

- **Menstrual phase:**
  increased expression IL-6, Il-1beta, ICAM-1, TGF-beta
  in women with endo > controls
- **Luteal phase:**
  increased expression TNF-alpha,MMP-3
  in women with endo > controls



Endometriosis =
Inflammation

PF
Nl peritoneum
EM (menstrual!)

Endometriotic lesion
Systemic

Ectopic lesion inflammation maintains local E2production (Bulun, 2009)

IL1beta →COX-2 → PG-E2 → SF-1 →aromatase →E2
VEGF → IL-1beta

E2→ ER-beta receptor→ COX-2

COX-2 elevated in
eutopic/ ectopic EM and PF macrophages
women with endo vs controls
(Ota et al, 2001; Chishima et al, 2002; Wu et al, 2002)

Endometriosis = *Subclinical Systemic Inflammation*

Endometriosis: Increased serum levels of:

- **CRP and SAA (Abrao, 1997)**
- **TNF-alpha (Bedaiwy, 2002; Pizzo, 2002)**
- **MCP-1, chemotactic and monocyte activating factor (Akoum, 1996; Pizzo, 2002; Gmyrek, 2005)**
- **CCR1-mRNA (PBL), receptor for RANTES, chemoattractant (Xu, 2005)**
- **Il-6 (Bedaiwy, 2002)**
- **IL-8 (Pizzo, 2002)**
- **sICAM-1 (Wu, 1998)**

BASIS FOR NONINVASIVE DIAGNOSTIC TEST?

IDEAL ANTI-ENDOMETRIOSIS DRUG

1. Prevent the development of endometriosis
2. Cures existing endometriosis, also after cessation of treatment
3. No interference with menstrual cycle
4. No side effects
5. Safe for women who wish to become pregnant

NON-HORMONAL ACTION OF ANTI-ENDOMETRIOSIS DRUGS

- **Reduce**

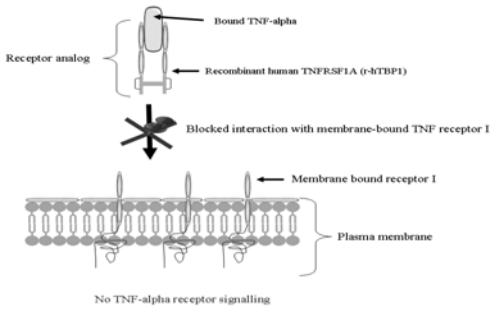
1. **Endometriosis-associated peritoneal inflammation: anti-TNF alpha, pentoxifylline, TZDs activating PPAR-gamma**
2. **Endometrial-peritoneal adhesion (anti-adhesion molecules)**
3. **Endometrial-peritoneal angiogenesis (anti-angiogenesis) Atorvastatin**
4. **Endometrial-peritoneal invasion (anti-proteases)**

? Alternative strategy: promote immune surveillance: increase macrophage activity/NK cell activity

Role of cytokines in endometrial-peritoneal attachment and invasion

- *In vitro* incubation of endometrial stromal cells with increasing concentrations of IL-8 has been reported to stimulate their adhesion to fibronectin (Garcia-Verlasquez, 1999)
- TNF-alpha can promote the adhesion of human EM stromal cells to peritoneal mesothelial cells (Zhang et al, 1993).
- Neutralization of TNF-alpha activity with anti-human Tumor Necrosis Factor –alpha can prevent or treat endometriosis in rodents and in baboons (Barrier et al, 2004; D'Hooghe et al., 2005,Falconer et al., 2006)

Therapeutic potential on inhibition of TNF-α



• TNFR1 functions by signaling for apoptosis (activation of caspases) and inflammation (activation of NF-κB and inflammatory gene products)
Kyama *et al.,* 2008

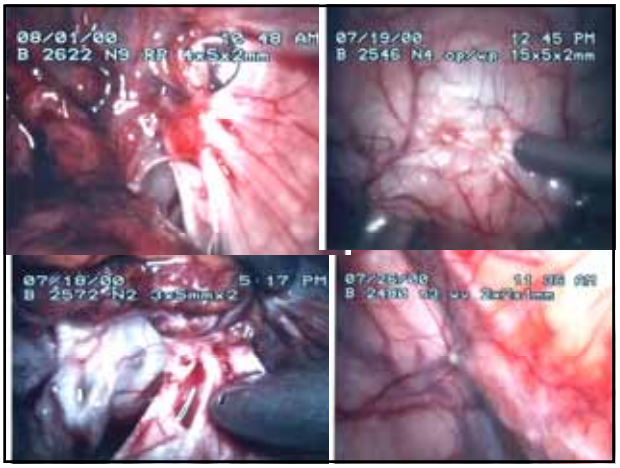
3 baboon studies

(Barrier et al, 2004; D'Hooghe et al, 2005; Falconer et al, 2006):

Inhibitory effect of TNF alpha antagonists mainly on active red peritoneal lesions

If confirmed in women, anti-TNF-α:

- first effective medical treatment of peritoneal endometriosis allowing ovulation (and conception if safe?)
- prevent progression to severe/deep disease ?
- prevent recurrence and onset of new disease?



TNFalpha inhibitors in women with endometriosis

- Only studied in 21 women with severe deep endometriosis-associated pain and rectovaginal nodule of at least 1 cm diameter awaiting surgery (Koninckx et al, 2008)
- Placebo-controlled RCT 2:1 (14 infliximab; 7 placebo)
- 12 week treatment period followed by surgery
- Outcome: 25-30% decrease in pain in both groups; no difference between both groups
- Comment: ? Inappropriate patient selection (TNFalpha inhibitors do not work well in fibrotic IBD)

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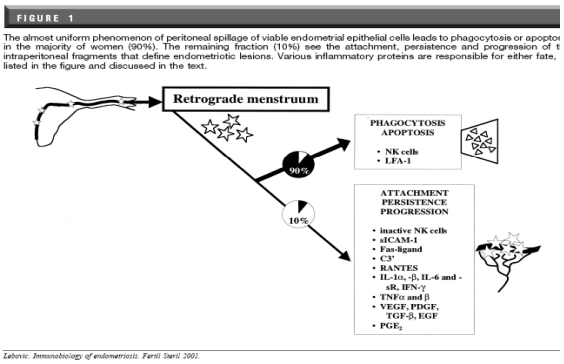
- Endometrial/Peritoneal inflammation
- Immune tolerance/autoimmunity

Why do not all women develop endometriosis?
Immune tolerance



Endo =Immunological tolerance versus self tissue

- I**mmunologically <u>specific</u> (**deletion or inactivation of antigen-specific T and/or B lymphocytes in thymus)
- **<u>Tolerance to self is learned or acquired</u>**.
- **Maintenance of tolerance requires <u>continuous availability</u> of tolerogenic antigens** to interact with immature lymphocytes

? Endo = Immune deficiency with decreased clearance of PF EM cells (1)

- Why would viable EM cells be a target for NK cells or macrophages? cfr autotransplantation of blood vessels, muscle, skin grafts, ...
- No in vitro or in vivo evidence that PF macrophages or NK cells actually attack and perform phagocytosis of viable PF EM cells

? Endo = Immune deficiency with decreased clearance of PF EM cells (2)

- Do women or female baboons have decreased NK activity in peripheral blood and decreased cytotoxic activity against autologous EM cells? CONTROVERSIAL (D'Hooghe and Hill, 1997)
- Increased PF and PB macrophage activity Endo > Co
- High dose immunosuppression may increase the progression of spontaneous endometriosis in baboons (D'Hooghe et al, 1995),but there is no clinical evidence that prevalence of endometriosis is increased in immunosuppressed patients
- Anti-inflammatory therapy prevents and reduces the development of endometriosis in animal models

? Are antigens on PF endometrial cells presented to T cells?

- Specific IR could occur if EM cells would present abnormal antigens together with MHCII to CD4 positive T lymphocytes
- Wallace et al, 2001 (in vitro)
- EM epithelial cells constitutively express MHC II, upregulated by IFgamma
- EM epithelial and stromal cells can process and present tetanos toxoid recall antigen driving autologous T cell proliferation

Endo: What do these T cells do in PF in vivo?

Tolerance in absence of costimulators?

- T cell Clonal anergy: CD4 T cells become unresponsive when they recognize antigens that are processed and presented by other human T cells expressing class II molecules but not the obligatory costimulators.
- In vivo : T cell clonal anergy after administration of large doses of protein antigens in aqueous solutions (ie endometrial antigens) , in the absence of adjuvants (costimulators)

Endo and Th2 immune response

- Th1 cells:

- pro inflammatory cytokines: IL2, IFgamma
- increased activity NKs, Macrophages, CD8 Ts
- synthesis of TNFalpha, VEGF, MMPs,..

- Th2 cells:

- anti inflammatory cytokines: IL4, IL10
- activation of B cells with Ig production
- activation of eosinophiles, basophiles, mast cells

Endo: Th2 IR component?

- PB (Antsiferova et al, 2005):
  increased proportion of CD4 cells
  + for IL4 and IL-10 (Endo > Controls)
- PF (Podgaec et al, 2007):
  Shift of PF cytokines towards Th2
  (predominance of IL4 and IL10 over IFgamma and IL-2)

Role of HLA-G in maintenance of immune tolerance of endometriosis

- Barrier et al, 2006

HLA-G: only expressed in

Peritoneal Endo: glandular epithelium (not stroma), Never in eutopic EM

HLA –G also expressed by mucosal epithelia in transplantation allografts

Role of HLA-G in maintenance of immune tolerance of endometriosis (Barrier et al, 2006)

-HLA-G expression by endometriotic lesions may protect against nl NK cell cytotoxicity

- Ectopic EM also benefits from other protective mechanisms: secretion of IL-10, EM haptoglobin, expression of Fas Ligand, sICAM-1, Complement regulatory factors (DAF, MCP), induction of anti-apoptotic proteins like bcl-2

Role of HLA-G in maintenance of immune tolerance of endometriosis (Barrier et al, 2006)

- Endo lesions surviving in context of chronic inflammation may stimulate weakly self-reactive lymphocytes → autoimmunity
- Formation of AE Abs:
  often against specific carbohydrate epitope: Thomsen-Friedenreich (T) Ag
- HLA-G expression by endometriotic lesions may represent an attempt to escape this anti-endometrial adaptive immune response

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Auto immunity

- Failure of mechanisms responsible for self-tolerance
- Anatomic alterations:

  - exposure to Ags that are normally sequestered and concealed from the immune system (posttraumatic uveitis, post-vasectomy orchitis)

  - unlikely in the case of endo: EM normally not concealed from the immune system

Common characteristics between autoimmune diseases and endo (Nothnick et al, 2001)

- Tissue damage
- Polyclonal B lymphocyte activation
- T-lymphocyte immunological abnormalities
- B-lymphocyte immunological abnormalities
- Altered apoptosis
- Multiorgan involvement (repro, bowel, uro)
- Preponderance of females
- Familial occurrence/possible genetic basis
- Possible environmental factors (dioxin?)
- Associated immune diseases

Endo-associated immune diseases (Sinaii et al, 2002)

EA, 3680 members, survey analysis

- Hypothyroidism
- Fibromyalgia
- Chronic Fatigue Syndrome
- RA, SLE, Sjogren, MS
- Allergies
- Asthma

Endometriosis and autoantibodies

- Nl human Auto Abs
  - low amount and low affinity IgMs
  - not generated with Tcell help
  - no tissue injury
  - stimulated (low levels) during IR to foreign antigens
- Pathological Auto –Abs
  - high amounts, high affinity Abs
  - help from autoreactive Tcells
  - tissue damage

ANTIBODY FEEDBACK:

- Abs: eliminate and neutralize antigens → remove initiating stimulus of the immune response
- 2 mechanisms:

formation of Ag-Ab complexes

C system activation

What is an Ab-mediated disease?

- 1. Tissue level: presence of Antibodies, C and/or ICs
- 2. Circulation level: Anti-tissue Abs or ICs, increased C activation products
- 3. Clinicopathological similarities with experimental diseases that are proved to be Ab-mediated by adoptive transfer

Ab-mediated tissue injury

- Complement-mediated cell lysis
- C5a and C3a mediated recruitment and activation of inflammatory cells at site of Ab deposition
- Phagocytosis of Ab-coated cells by phagocytes carrying Fc receptors and C3b receptors
- Lysis of Ab-coated cells by Fc receptor carrying NK cells
- Ab binding to functionally important molecules
- Ab altering cell functions

Endo = not an Ab-mediated disease?
Abs, ICs and C in EM and in EL

- Endogenous IgG (IgA, IgM): present in EM (but not specific for Endo) and in EL
- Complement:
- no evidence for C activation in EM from women with endo
- EL: inhibition of C activation protecting EL glandular cells from autologous complement attack in vivo

Endo = not Ab-mediated disease?
Ig and C in PB or PFcirculation

- IgG, Ig A or IgM: endo = controls
- C: endo = controls ,but studies needed with better measurement of C activation:
- C3 split products (C3b, C3c, C3d)
- C4
- MAC

Auto Abs against phospholipid Ags, histones and nucleotides in PB/PF

- Ag independent stimulation of self-reactive clones not deleted during development
- By polyclonal activators
- Production of multiple Auto Abs-systemic effects
- May lead to IC-mediated disease: fibrinoid necrosis and neutrophil infiltration (SLE)
- No evidence of that prevalence or levels of these Abs are higher in Endo than in Controls.

Anti Endometrial Auto Abs

- ANTIGEN MIMICRY
  Immunological cross-reaction between foreign -self Ags: IR induced by foreign Ag, also directed against self Ag
- Auto-Abs against self tissues: not always pathogenic
- AEAbs: Endo> Controls (Fernandez-Shaw, 1993; Kim et al, 1995; Ibora, 2000),
  positively correlated with Endo severity
- ? Against Ags with MW 26, 34, 42
- ? Secondary effect after induction of endo (rabbits, Homm et al, 1989)

AE Abs specific for Thomson-Friedenreich-like Carbohydrate Ag

- Lang and Yeaman, 2001:
- Defined Anti Carbohydrate AutoAb response is present in Endo sera
- Recognized epitope is carried on Endometriosis Auto Ags like:
  Alpha-HSG, Carbonic Anhydrase II, Hemopexin, and IgA
- Higher prevalence of AutoAbs against these Ags in PB/PF of endo > controls

Endo = autoimmune disease?

Update of theory proposed by Dmowski et al, 1990

- PF EM cell antigens: processed by activated macrophages and presented to T cells
  Current view: direct presentation of Ags by EM cells to T cells
- Macrophage-released cytokines→ T cell proliferation and differentiation into functional subsets:
  helper, suppressor-inducer and cytotoxic
  Current view: direct EM Ag presentation to T cells leads to their proliferation
- T cell-derived factors →activation of B cells: differentiation and antibody secretion
  Current view: Th2 response leading to production of Abs

Endo = autoimmune disease?

Update of theory proposed by Dmowski et al, 1990

- Auto-Abs against endometrial cells or against endometrial cell-derived phospholipids, histones or nucleotides.
  Current view: AutoAbs against endometrium and against TF Ag (carbohydrate epitope)
- Autoantibodies : negative effect on fertility by interfering with ovum capture or implantation (serum markers?)
  Current view: no evidence (Valeo Medical), Auto Abs may be non-functional epiphenomenon

Endo = immune tolerance

- Quantity of retrograde menstruation/PF EM cells
- Inflammation: local (PF, EM, nl P, EL)+systemic
- HLA-G expression in glands of EL: protection against activity of NK, macrophage and AEAbs
- Activation via EL of weakly selfreactive T cells → Th2 response and formation of AEAbs.
- Endo = not Ab-mediated disease
- Function AEabs not clear, possibly epiphenomenon

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INSTITUTE OF PRIMATE RESEARCH
NATIONAL MUSEUMS OF KENYA
WHO COLLABORATING CENTRE

Collaboration

- Institute of Primate Research, Nairobi, Kenya, WHO Collaborating Center
- WHO
- University of Michigan, Ann Arbor, USA
- Oxford and Cambridge Universities, UK
- European Network Endometriosis
- Karolinska University, Stockholm, Sweden
- Semmelweis University, Budapest, Hungary
- Endometriosis Association, Milwaukee, USA
- World Endometriosis Research Foundation, London, UK

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- Leuven IRO (internatiole raad ontwikkelingssamenwerking): PhD Kyama
- Leuven KOF (klinisch onderzoeksfonds): Phd Meuleman
- Belgian Fund for Scientific Research (FWO)
- Belgian Institute for Science/Technology (IWT)
- Flemish Government (endocrine disrupters)
- Endometriosis Association USA
- University Michigan Ann Arbor USA
- World Endometriosis Research Foundation
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