Immunological Factors in Endometriosis

State of the art

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Group leader ‘Reproductive Sciences Lab’

San Raffaele Scientific Institute
Endometriosis development

Endometrial ability to implant

Immune surveillance

Vigano’, 1999
The 90’s: NK cell activity in women with endometriosis

1. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium.
   


3. Suppression of natural killer cell activity by sera from patients with endometriosis.


4. The natural killer activity of peritoneal fluid lymphocytes is decreased in women with endometriosis.

   Oosterlynck et al, Fertil Steril, 1992

5. Natural killer cell activity in endometriosis: correlation between serum estradiol levels and cytotoxicity.

   Garzetti et al, Obstet Gynecol, 1993


   Wilson et al, Fertil Steril, 1994

7. Peritoneal natural killer cytotoxicity and CD25+CD3+ lymphocyte subpopulation are decreased in women with stage II-IV endometriosis.

   Ho et al, Hum Reprod, 1996
8. Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis.

   \textit{Wu et al, Fertil Steril, 2000}

9. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression.

   \textit{Kang et al, Hum Reprod, 2014}

10. Platelet-derived TGF-\(\beta\)1 mediates the down-modulation of NKG2D expression and may be responsible for impaired natural killer (NK) cytotoxicity in women with endometriosis.

   \textit{Guo et al, Hum Reprod, 2016}

11. IL15 promotes growth and invasion of endometrial stromal cells and inhibits killing activity of NK cells in endometriosis.

   \textit{Yu et al, Reproduction, 2016}
Natural Killer cell cytotoxicity
Natural killer cell receptors

NK cells as key players in the initiation, promotion and progression of the disease

or

NK cell abnormalities only a consequence
Evidence from GWAS

- Cell-cell interaction
- Regulation of gene transcription
- Steroid response
- Matrix Remodelling/Cytoskeleton organization
Immunology of endometriosis: *state of the art*

**Eutopic endometrium women with VS without endometriosis**

- ICAM-1 ↓
- Nectin-3 ↑
- Nectin-4 ↑
- IL-6 ↑
- MCP-1 ↑
- IL-37 ↓
- Galectin-1, -3, -9 ↑
- CX3CR1 ↑
- Fractalkine ↑
- PIAS3 ↓
- TSG-6 ↑
- Semaphorin E ↑
- IL-15 ↓
- Glycodelin ↓
- FOXO1 ↓
- MAD2L1 ↓
- MUC-1 ↓
- Osteopontin ↓
- MIG6 ↓
- Toll-like receptor 3 ↓
- Toll-like receptor 4 ↓
- PR ↓
- NF-kBp65 ↑
Immunology of endometriosis: state of the art

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- MIG6 ↓
- Toll-like receptor 3 ↓
- Toll-like receptor 4 ↓
- PR ↓
- NF-kBp65 ↑
PROGESTERONE resistance

- Unbalanced estradiol action
- Enhanced tissue-adhesive processes
- Abnormal activity of metalloproteinases
- Incomplete endometrial decidualization
- Altered embryo receptivity

PROGESTERONE RESISTANCE IN EUTOPIE ENDOMETRIUM
Immunology of endometriosis: *state of the art*

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- Fractalkine ↑
- PIA53 ↓
- TSG-6 ↑
- Semaphorin E ↑
- IL-15 ↓
- Glycodelin ↓
- FOXO1 ↓
- MAD2L1 ↓
- MUC-1 ↓
- Osteopontin ↓
- MIG6 ↓
- Toll-like receptor 3 ↓
- Toll-like receptor 4 ↓
- PR
- NF-kBp65 ↑
Negative interaction between p65 subunit of NF-kB and the PROGESTERONE RECEPTOR

Mutual repression

Always consider the NF-kB engagement!!
Immunology of endometriosis: state of the art

Eutopic endometrium
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  - IL-6 ↑
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  - Osteopontin ↓
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  - Toll-like receptor 3 ↓
  - Toll-like receptor 4 ↓
  - PR ↓
  - NF-kBp65 ↑

Ectopic sites
- Local immune cell recruitment
  - Recruitment of alternatively activated macrophages
  - Recruitment of dendritic cells
  - Recruitment of T_{REG}, T_{H}
  - Production of chemokines - RANTES, MCP-1, IL-8
  - Immune-mediated vasculogenesis
  - DAMPs release

Ectopic tissue
- APOE
- PPARG
- C1R, C3, C7
- HLA-DRA1
- HLA-DRB1
- HLA-DQB1
- HLA-C
- HLA-G
- IL-15, IL15R
- TGF-β3
- IL18, IL18R1, IL18RAP5
- CCL5
- CCR2
- S100A8

Systemic response
- IL-6, IL-8, sICAM-1, sCD23, MCP-1, copeptin, CRP,
  - MIF, TNF-α, INF-γ, IL-1β, IL-4, glycodelin
- Anti-endometrial antibodies
IL-6 as diagnostic blood test
Gene expression profile of PERIPHERAL BLOOD MONONUCLEAR CELLS in patients with moderate-severe endometriosis

**Methods**
- matched PBMC samples before and after six months from an intervention for III-IV stage disease
- extraction of RNA, cDNA synthesis, hybridization to an Affymetrix U133 Plus gene array displaying 12000 known genes and 10000 expressed sequence tags
- Real-time PCR analysis of genes identified

**Results**
- N=26 genes up-regulated before the intervention
- N=15 genes down-regulated before the intervention

Gentilini et al, Hum Reprod, 2011
Gene expression profile of PERIPHERAL BLOOD MONONUCLEAR CELLS in patients with moderate-severe endometriosis

### Endometriosis

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>GenBank</th>
<th>Fold Change</th>
<th>p-value</th>
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### Psoriasis

<table>
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<tr>
<th>Gene Symbol</th>
<th>Accession Number</th>
<th>Fold Change</th>
<th>p-value</th>
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</table>

The highlighted genes resulted to be commonly up-regulated both in endometriosis and in psoriasis condition.

5.2 x 10^{-11} hypergeometric probability

Gentilini et al, Hum Reprod, 2011
Gene expression profile of PERIPHERAL BLOOD MONONUCLEAR CELLS in patients with moderate-severe endometriosis

- Endometriosis induces the expression of genes in peripheral leukocytes already identified in non-gynaecologic chronic inflammatory diseases, thus revealing the disease as a local affliction with relevant consequences at the systemic level.

- The surgical intervention may ‘switch off’ the expression of these genes.
Reduced NK cell cytotoxicity

- IL-15
- Granzyme B
- IFN-γ
- Endometrial cells
- TGF-β1
- Platelets
- NKp44
- NKG2D

NK CELLS

Reduced NK cell cytotoxicity
Modulation of NK cell lytic function by endometrial secretory factors

Acquired immune dysfunction!

- Reduced NK cell cytotoxicity

**NK CELLS**
- IFN-γ
- Granzyme B
- Perforin

**Platelets**
- TGF-β1

**Endometrial cells**
- IL-15

**macrophages**
- IL-6

**NK cell receptors**
- NKG2D
- NKp44

**Signaling molecules**
- SHP2
NK cells and mouse models of endometriosis

**NK CELL SUPPRESSION**

| Table 1. Take Rates of Human Endometrium in SCID Mice, Anti-Asialo GM₁ Antibody-Treated Nude (Ab-Nude) Mice, and Nontreated Nude (NT-Nude) Mice |
|---|---|---|---|---|
| Group | 2 wk | 4 wk | 6 wk | 8 wk |
| SCID mice | 10/10 | 10/10 | 10/10 | 10/10 |
| Ab-nude mice | 10/10 | 10/10 | 10/10 | 10/10 |
| NT-nude mice | 8/10 | 8/10 | 7/10 | 5/10³ | 4/10⁴ |

* No. of mice with surviving graft/no. of mice grafted.

† P < .05 vs other groups, χ² test.

‡ P < .01 vs other groups, χ² test.

**NK CELL DEPLETION**

Aoki et al, Obstet Gynecol, 1994

Guo et al, Hum Reprod, 2017
Conclusions

• Consistent evidence strongly supports a dysfunction of NK cells in women with endometriosis. Whether this is an acquired or primary alteration remains to be established.

• Endometriosis has some consequences at systemic level on genes already involved in chronic inflammatory diseases.

• A consequence of the inflammatory environment (i.e. NF-kB pathway) might be envisaged when dealing with alterations of eutopic or ectopic endometrium.

• Results from GWAS do not support a crucial role of the immune system in the predisposition to the disease.
Thanks

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