

CASE PRESENTATION

A 42 year old woman presents with a 4 year history of progressive abdominal pain.

She is nulligravid intentionally, a career woman, never used oral contraceptives.

She initially noted the pain in her lower abdomen, occasionally accompanied by increased urinary frequency and urgency but of late the pain is diffuse and occasionally she feels it in her shoulders

It has gotten much worse during her periods.

Her cycles are every 28-32 days lasting about 4 days with no intermenstrual spotting.

She had seen a doctor for this about 3 years ago but the doctor wanted to do some sort of surgery and she had no time to be bothered with this so she took NAPROSYN and found she had been getting good relief until about a year ago.

What is the next step ?

Endometriosis

In ovarian tissue
cysts often develop
as a result of
proliferation and
bleeding into the
ovary

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Endometriosis

Areas of
endometriosis
are
characteristically
cystic and often
hemorrhagic

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Five Critical Steps

1. Attachment of endometrial cells to the peritoneal surface
2. Invasion of these cells into the mesothelium
3. Recruitment of inflammatory cells
4. Angiogenesis around the nascent implant
5. Endometrial cellular proliferation

Endometriosis

- Monkeys are only animal (cyclic)
- No cases reported prior to puberty
- Often seen in teenage years
- Short cycle and longer flows have twice the risk of endometriosis
- Early menarche
- Delayed childbearing
- Menstrual outflow obstruction

Retrograde Menstruation Theory

- Implantation (Sampson's theory)
- Viable endometrial tissue is refluxed through the fallopian tubes during menstruation
- Implants on peritoneal surface or pelvic organs

Model of Endometriosis Development

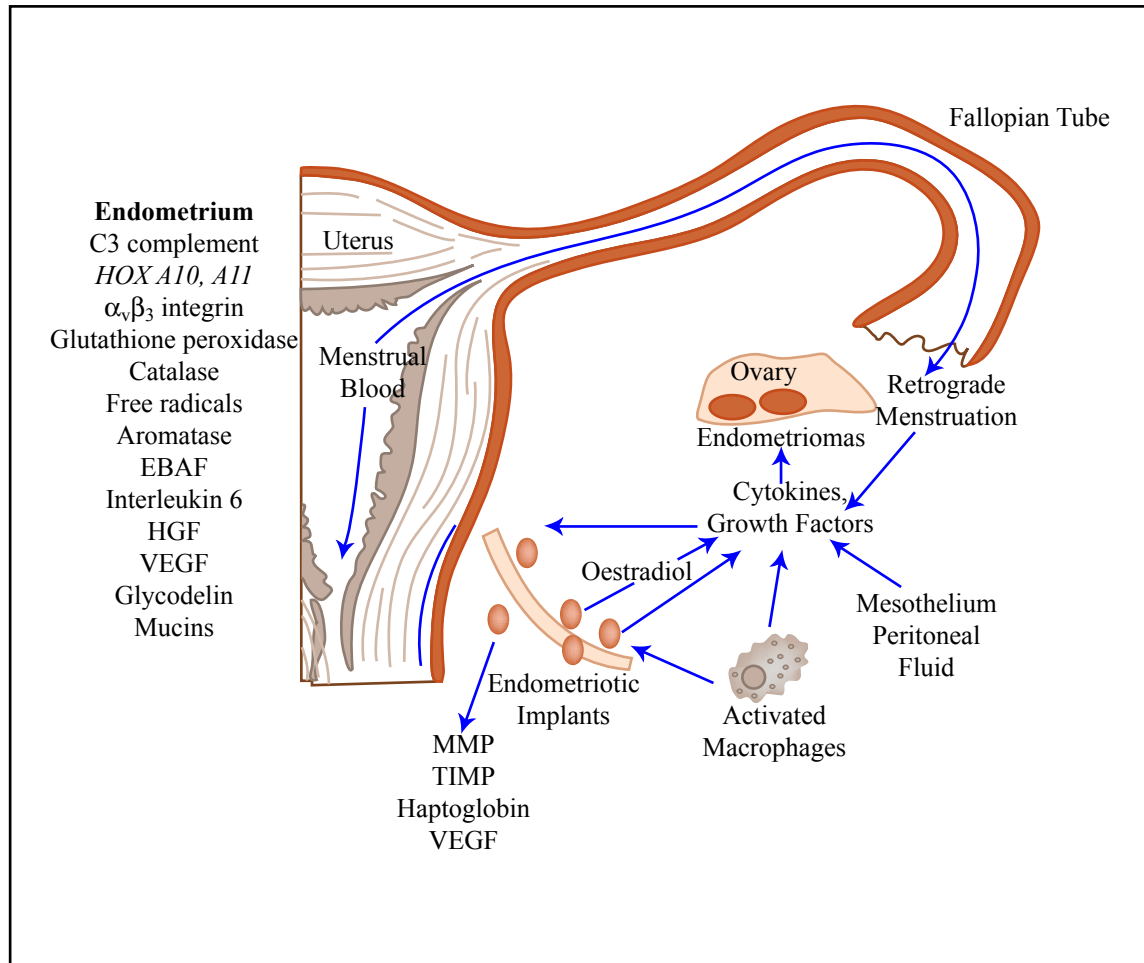


Figure by MIT OCW.

Retrograde Menstruation

- 1938 - Watkins observed blood dripping from fallopian tubes in women who underwent laparotomy during menstruation
- Goodall reported that retrograde menstruation occurred in 50% of women who underwent laparotomy during menstruation
- Presence of blood in the peritoneal fluid was also observed in women who underwent peritoneal dialysis
- Retrograde menstruation is a common phenomenon that occurs in 76% to 90% of women with patent fallopian tubes

Viability

- 1951 - Keetel and Stein cultured endometrial cells obtained from menstrual discharge of seven women who wore diaphragms
- Endometrial cells obtained from peritoneal fluid after uterine lavage also were cultured successfully
- Endometrial cells collected from the peritoneal cavity after uterine lavage stayed viable in culture for up to 2 months
- Endometrial cells obtained from peritoneal fluid also were cultured successfully

Adherence

- 1950 - Scott and TeLinde reported that shed endometrial cells were able to implant
- In monkeys shown that 50% of the monkeys developed endo
- Baboons - after injection of menstrual endometrium into their retroperitoneal space
- Menstrual effluent from women during the second day of menstruation - injected it into the subcutaneous abdominal fat of patients → viable endometrial glands and stroma

Retrograde Menstruation Theory

● Assumptions

- ***Retrograde menstruation*** occurs through the fallopian tubes
- Refluxed endometrial cells are ***viable***
- Refluxed endometrial cells are able to ***adhere*** to peritoneum

Clinical Data

- Increased risk of endometriosis in patients with Mullerian anomalies and obstructed flow
- Increased frequency of endometriotic implants in the dependent areas of the pelvis

Endometriosis

- Implantation & Metastases
 - Menstrual effluent (Sampson)
 - Retrograde menstruation
 - Common event
 - Viable endometrial cells noted
 - Why does a physiologic event yield pathology ?
 - Antegrade cells cultured and contain adhering and proliferating cells that are either epithelial or fibroblastic in appearance (Keettel & Stein)

Endometriosis of Uterus

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Endometriosis

- Coelomic Metaplasia
 - Ovary & mullerian ducts derive from coelomic mesothelium
 - Germinal epithelium attempts to recapitulate endometrium
 - Only explains ovarian endometriosis
- Peritoneal mesothelium is **totipotential**

Coelomic Metaplasia - Meyer

- Develops from metaplasia of cells that line the pelvic peritoneum
- Infectious, hormonal, or other inductive stimuli may result in metaplasia

Derivatives of Epithelium of the Coelomic Wall

- Pelvic peritoneum
- Germinal epithelium of ovary
- Mullerian ducts
- Examples
 - Ovarian surface endometriosis
 - Men (undergoing estrogen therapy for prostate cancer)
 - Prepubertal
 - Adolescent girls
 - Women who never menstruated
 - Unusual sites - pleural cavity (? Trans-diaphragmatic ?)

Induction Theory

- Extension of the coelomic metaplasia theory
- Endogenous biochemical or immunologic factors - induce differentiation into endometrial tissue
- Supported by observations in female rabbits
- Implanted sections of uterine wall – stimulated development of endometriosis
- Millipore filters that contained myometrium, fat, or endometrium → Implants were later excised with the surrounding tissue and examined histologically.
- In vitro coelomic metaplasia in ovarian surface epithelium co-cultured with endometrial stromal cells in high estrogen environment

Endometriosis

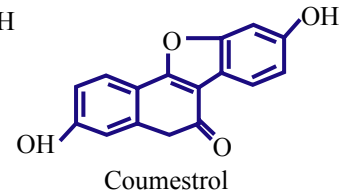
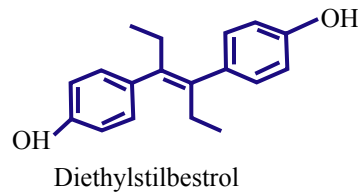
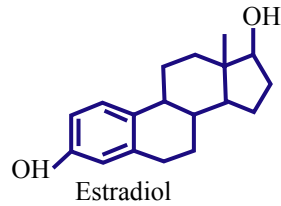
● Dissemination

- Disseminated tissue can cause metaplasia
- Injection into ear vein of rabbit causes endometriosis of lungs
- Laparotomy scar
- Episiotomies
- Cesarean sections
- Transplantation confirmed in animal experiments

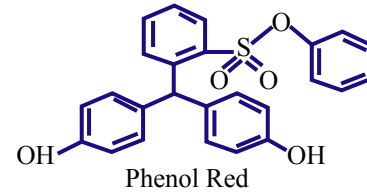
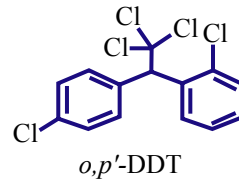
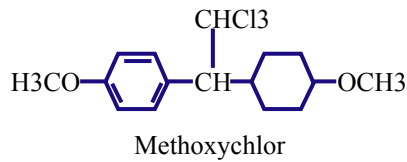
Other Theories

- Embryonic rest theory
- Cell rests of Mullerian origin
- Lymphatic and hematogenous dissemination of endometrial cells
 - Evidence suggests that endometrial cells can metastasize
 - Pleura, umbilicus, retroperitoneal space, lower extremity, vagina, and cervix - are anatomically possible
 - Endometrial tissue in uterine veins in women with adenomyosis
 - Induced pulmonary endometriosis by injecting endometrial tissue intravenously in rabbits
 - Lymph node endometriosis was found to be present in 6.7% autopsies

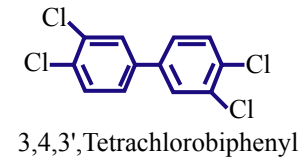
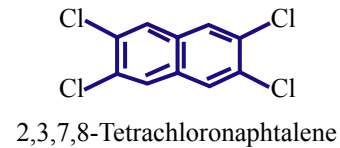
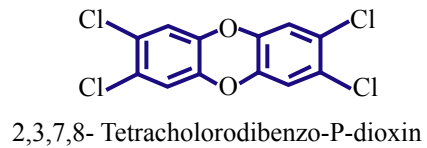
ESTROGENIC COMPOUNDS



Natural and Synthetic Estrogens



Estrogenic Compounds



Polyhalogenated Aromatic Compounds (Dioxin and Dioxin-like Compounds)

Figure by MIT OCW.

Environmental Factors

- Exposure to environmental toxins
- Prototype standard 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
- Member of family of polychlorinated diaromatic hydrocarbons
- Reference compound for effects of all other polychlorinated diaromatic hydrocarbons.
- Lipophilic property - degrade slowly - accumulate in the food chain
- Exposure of TCDD mostly through ingestion of contaminated foods
- Exert their effects via aryl hydrocarbon receptor
- Orphan nuclear receptor whose natural ligand is not known
- Receptor is present in many tissues, including eutopic and ectopic endometrium

Environmental Factors

- **The non-human primate model**
 - **Rhesus monkeys exposed to whole-body proton irradiation**
 - higher frequency of endometriosis (53% vs 26%)
 - **Rhesus monkeys exposed to 5–25 ppm dioxin per day for 4 years developed endometriosis**
 - dose-dependent
- **Extrapolation to women was initially thought to be epidemiologically plausible**
 - **Belgium - highest dioxin pollution in the world**
 - Highest incidence of endometriosis
 - Highest prevalence of severe endometriosis
 - **Two subsequent prospective studies from Italy and Belgium**
 - No significantly increased risk of endometriosis
- **Multiplicity of chemicals**
- **Mechanisms of action that might vary**
- **Dose**
- **Timing of exposure (in utero, childhood, peripuberly, adult)**
- **Route of exposure**
- **Synergy with other chemicals**

Environmental Factors

- Can inhibit ovarian progesterone synthesis
- Inhibits progesterone-induced expression of TGF- β that suppresses endometrial MMPs
- Endometriosis spontaneously developed in monkeys exposed to dietary TCDD
- Size of the implants was found to be significantly increased with exposure
- Infertile women with endometriosis compared to women with tubal infertility are more likely to have a history of TCDD exposure !!!!!

Proton Irradiation

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Endometriosis

UMBILICUS

- Less frequent site
- Dramatic
- Patient bleed from umbilicus every month

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Endometriosis

- ABDOMINAL WALL
- Removed at time of cesarean section
- Decidual reaction

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Endometriosis

- **INTESTINAL TRACT**
- Terminal ileum
- Sigmoid colon
- Appendix

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Endometriosis - Terminal Ileum

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Endometriosis in Colon Wall

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GI IMPLANTATION SITES

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ENDOMETRIOSIS OF BLADDER

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Rare Sites of Endometriosis

- Bone
- Muscle
- Brain
- Nerve
- Lung parenchyma
- Vertebral space
- Extremities

Genetics of Endometriosis

- 1971 questionnaire survey - Ranney
- 1980 First formal study - Simpson
- 123 probands with endometriosis
 - 9 (5.9%) female siblings ov 18 + for endo
 - 10 of the 123 (8.1%) mothers were affected
- Women with an affected sibling or parent - more likely to have severe than mild or moderate endometriosis

Genetics of Endometriosis

- A 5–8% risk for first-degree relatives is more consistent with polygenic/multifactorial tendencies
- Studies of shared genes indicate lower risk with fewer shared genes
- Increased severity in individuals with higher risk of developing endometriosis
- Need only a few genes to show continuous variation

Genetics of Endometriosis

- Proposed that endometriosis originates through a series of multihits within target genes
- Similar to cancer
- Typically comprised of
 - Point mutations
 - Rearrangements
 - Duplications
 - Deletions
- Genes of special interest
 - Oncogenes
 - Tumor suppressor genes

Genetic Factors

- Study One
 - 8.1 % of their mothers and 5.9% of their female siblings older than age 18 were affected
 - 1 % of the controls had endometriosis
- Study Two
 - 3.9% of mothers and 4.8% of sisters with endometriosis had endometriosis 0.6% of sisters of controls had endo
- Study Three
 - 6.2% and 3.8% incidence in sisters and mothers
- Study Four
 - 8.6% of first-degree

GENETIC FACTORS (summary)

- Much more common in patients with a FH
- Maternal inheritance pattern
 - 7% in first degree relatives
- More severe in women with a + first degree relative
- 6/8 monozygotic twins had endometriosis
- 3.8% of non-monozygous sisters
- Polygenic/multifactorial
- No HLA system seems involved
- Perhaps different diseases (multiple genes)

CLINICAL ASSESSMENT

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EUTOPIC vs. ECTOPIC ENDOMETRIUM

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Endometriosis & Pain

- Severe pelvic pain is often associated with endometriosis
- Pain can be diminished with therapies that suppress estrogen production
- Many women also suffer from other chronic pain conditions
- Involve the growth into the ectopic endometrial tissue of a nerve supply
- Widespread influence on the activity of neurons throughout CNS

Endometriosis & Pain

- Endometrial implants respond to natural or induced decreases in estrogen levels
- Reduced fertility and several types of pain
 - Severe dysmenorrhea
 - Deep dyspareunia
 - Dyschezia
 - Chronic pelvic pain
- Can be exacerbated by the co-occurrence
 - Irritable bowel syndrome
 - Interstitial cystitis
 - Repetitive kidney stones
 - Vulvodynia
 - Temporomandibular fibromyalgia

Endometriosis & Pain

- Most common pharmacological treatment = gonadotropin-releasing hormone (GnRH) agonists
- Down-regulate GnRH receptors
- Suppress pituitary gonadotropin secretion and sex steroid production
- Produce systemic hypoestrogenic state
- Elimination or reduction in size of the implants
- Reduces endometriosis-related pain symptoms
- Failed to find a correlation among
- pain scores
- Types of pain
- Anatomy
- Biochemistry of the implants

Endometriosis & Pain

- Surgical removal of the ectopic usually implants alleviates pain symptoms
- Surgery can fail to alleviate the pain
- Pain may recur even without evidence of residual or recurrent disease
- No other identifiable visceral or somatic pathology

Endometriosis & Pain

● Correlations

- Pain severity
- Depth of "infiltration" into peritoneum or pelvic organs
- Substances released into the tissue or peritoneal fluid
 - Proinflammatory cytokines
 - Prostaglandins
 - Chemokines

● Patients reporting pain

- Deeply infiltrating implants
- In highly innervated areas
 - Utero-sacral region
- Nerve fibers are closer to the implants

Endometriosis & Pain

Rat Model

- Valid for studying ectopic implants, subfertility, pain
- Respond similarly to hormonal treatment
- Show similar alterations in protein production
- Rats - subfertile
- Do not exhibit spontaneous pain behaviors
- Develop an increased pain sensitivity
 - Vagina
- Severity correlates with estradiol levels
- Similar to dyspareunia in women
- Urinary bladder capacity is reduced
 - Similar to interstitial cystitis

Endometriosis & Pain

Rat Model

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Endometriosis & Pain

Rat Model

- Ectopic implants develop a sensory sympathetic supply
 - Similar to healthy uterus
 - In rats the supply is connected to CNS via vagus
 - Same input as from ureters
 - Sensory fibers of type seen in rat are activated and sensitized by many inflammatory agents
 - Variability of inputs to CNS and variability of sensitization by estrogen ? mechanism

Attachment

- Endometrial fragments obtained in either phase of the cycle – adhere to the epithelial side of the amnion but only at locations where the amniotic epithelium was damaged or absent
- Cultured peritoneal explants adhered to peritoneal explants only at locations where the mesothelium was absent or damaged and the basement membrane was exposed
- Intact mesothelium constitutes a defense barrier
- Occasionally there is attachment to intact mesothelium

Attachment

- Peritoneal mesothelium produces *hyaluronic acid*
- Hyaluronic acid is expressed along the cell membrane and contributes to the pericellular matrix
- Major component of the extracellular matrix ground substance
- *CD44* is the principal receptor for hyaluronic acid
- Involved in binding of gastric cancer and ovarian cancer cells to mesothelium
- Endometrial stromal and epithelial cells express CD44
- Hyaluronidase pretreatment of mesothelial cells decreases the binding of endometrial stromal and epithelial cells to mesothelium

INFERTILITY

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Endometriosis of Fallopian Tube

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PERITONEAL FLUID

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Immune System's Role

- Believed to be involved in the pathogenesis of endometriosis
- Lack of adequate immune surveillance in the peritoneum
- Evidence of activation of peritoneal macrophages
 - Increased cytokine production
 - Although there is **decreased** phagocytic activity
- Using the approach of two-dimensional gel electrophoresis
 - Protein called Endo I in endometriotic epithelial cells
 - Not observed in eutopic endometrial epithelium
 - Structurally similar to haptoglobin
 - Bound to peritoneal macrophages
- Macrophages increased their production of interleukin 6
 - Reduced macrophage phagocytic capacity
 - Interleukin 6 upregulates endometriotic production of Endo I
 - ? role for haptoglobin in the pathogenesis of compromised immune surveillance
- Potential targets for therapies for pain and infertility by inhibition of haptoglobin's actions
- Evidence of compromised natural-killer-cell activity in peritoneal fluid
 - May lead to decreased immune surveillance of ectopic tissue

Immunologic Factors

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Surgical Management

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Theoretical Model of Endometriosis

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Candidate Genes

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Aberrant Genes & Gene Products

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Theories Regarding Development of Endometriosis

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Attachment to Mesothelium

- Amniotic membrane vs peritoneum with respect to expression of cytokeratins in epithelial lining
- Endometrial fragments do not adhere to the epithelial side of the amniotic membrane
- Adhesion does occur on the non-epithelial side
- **Intact** epithelial lining may prevent initial adhesion of retrogradely shed endometrium

Adhesion Molecules

- Integrins
- Intracellular adhesion molecule-1
- Vascular cell adhesion molecule-1
- Integrin-blocking antibodies do not interfere with endometrial stromal or epithelial cell adherence to mesothelium

Matrix Metalloproteinases

- Invasion follows initial adhesion
- Matrix metalloproteinase (MMP) enzymes - implicated
- MMPs (and inhibitors) play a significant role in normal endometrial remodeling that accompanies menses
- MMP family contains several structurally related Zn^{2+} -dependent endopeptidases
- Responsible for the degradation of various extracellular matrix components

Matrix Metalloproteinases

- collagen
- gelatins
- proteoglycans
- laminin
- fibronectin
- elastin

Matrix Metalloproteinases

- Increased MMP activity in and around the endometriotic implants may facilitate invasion and growth of lesions.
- Progesterone down regulates endometrial MMP expression

Transforming growth factor- β (TGF- β)

- Produced by endometrial tissue in response to progesterone
- TGF- β suppresses expression of MMP-7
- Antibody to TGF- β abolishes this suppression
- Blocking the action of TGF- β opposes progesterone-mediated suppression of MMP-3 and MMP-7
- Blocks the ability of progesterone to prevent experimental endometriosis

Transforming growth factor- β (TGF- β)

- TGF- β alone does not lead to sustained suppression of MMPs
- Possibly because of resumption of MMP production in the absence of progesterone
- Consistent with the fact that peritoneal fluid levels of TGF- β are elevated in endo

Interleukin-1 (IL-1 α)

- Potent stimulator of MMP-3 in proliferative phase endometrium
- Progesterone exposure in vivo reduces the IL-1 α stimulation of MMP-3 in secretory phase tissue
- IL-1 α stimulation of MMP-3 is restored in a dose-dependent manner with progesterone withdrawal
- Cultured endometriotic cells obtained from a rat endo model express higher levels of MMP-3 mRNA than eutopic rat endometrial stromal cells when treated with progesterone
- Elevated and persistent MMP-3 expression by endometriotic stromal cells cultured in the presence of progesterone correlates with elevated levels of IL-1 α mRNA detected in the endometriotic stromal cells
- Production of IL-1 α by the endometriotic lesions - overcomes the progesterone-induced suppression of MMP-3
- IL-1 α - related mechanism promotes MMP-3 production by endometriotic cells even in the presence of progesterone

Impaired Immunity

- Impaired immune response - inadequate removal of refluxed menstrual debris
- Endo associated with changes in cell-mediated and humoral components
- Peritoneal fluid of women with endo contains increased numbers of immune cells
- Facilitate rather than inhibit the development of endometriosis
- Unclear if immunologic alterations induce endo or are a consequence

Pelvic Inflammation

- Contributes to pain and infertility
 - Cytokines
 - Prostaglandins
 - Dyspareunia
 - Chronic pelvic pain
- Inflammation --> Infertility
 - adhesion formation
 - scarring
 - disrupt fallopian tube patency
 - impair folliculogenesis
 - fertilization
 - embryo implantation

Macrophages

- Most abundant nucleated cells found in peritoneal fluid
- Increased in the peritoneal fluid of women with endometriosis
- Promotes growth of ectopic endometrium (paradox ?)
- Increase in the release of growth promoting cytokines

Impair Scavenger Function

- Abnormal levels of cytokines present in the peritoneal fluid
- Lack of interaction between macrophages and extracellular matrix
- Results in a decreased expression of scavenger receptors
- Cause the decrease in scavenger function
- Secretory products of peritoneal macrophages and circulating monocytes mediate growth and maintenance of ectopic endometrium
- Peritoneal fluid stimulates proliferation of cultured endometrial stromal cells
- Peripheral blood monocytes of co-cultured autologous endometrial cells
Monocytes from fertile women suppress endometrial cell proliferation
- Implicated in the pathophysiology of endometriosis associated pain and infertility

Natural Killer Cells (NK)

- Decrease in NK cell activity may lead to impaired clearance of regurgitated endometrial cells from the peritoneal cavity
- Decreased cytotoxic activity against autologous and heterologous endometrium
- More pronounced in the moderate and severe stages of endo
- Sera and peritoneal fluid from women with endo suppress NK cell cytotoxicity Higher killer-inhibitory receptors expression
- Send inhibitory signals that override the kill signal and suppress cytotoxic activity

Lymphocytes

- T-cell mediated immunity to autologous endometrium is suppressed
- Cytotoxic activity of peripheral blood lymphocytes against autologous decreased
- Functional alteration not accompanied by a quantitative down regulation
- Total in the peripheral blood are not affected markedly
- No change in total lymphocyte content or helper/suppressor ratios
- T-lymphocyte concentration is increased in the peritoneal fluid

Endocrine Factors

- Endometriosis is an estrogen-dependent disorder
- Aberrant estrogen synthesis and metabolism –
 - Aromatase catalyzes the synthesis of estrone and estradiol from androstenedione and testosterone, respectively
- Expressed by many human cell types
 - Ovarian granulosa cells
 - Placental syncytiotrophoblasts
 - Adipose cells
 - Skin fibroblasts
- Estrogen produced by aromatase activity in the cytoplasm of leiomyoma smooth muscle cells or endometriotic stromal cells
- Disease-free endometrium and myometrium lack aromatase expression

Endometriomas and extra-ovarian endometriotic implants express high levels of aromatase

- Cultured stromal cells derived from endometriotic implants and incubated with a CAMP analog display extraordinarily high levels of aromatase
- Growth factors, cytokines - possible inducers of aromatase
- Prostaglandin E₂ was identified as the most potent inducer
- Estrogen - up-regulates prostaglandin E₂ formation
- Stimulates cyclo-oxygenase type 2 enzyme in endometrial stromal cells
- Positive feedback loop for continuous local estrogen and prostaglandin E₂ production
- Possible genetic defect in aromatase expression in endo

Possible role of Aromatase in Endometriosis

- Androstenedione of adrenal and ovarian origins – premenopausal women
- Adrenal androstenedione in postmenopausal women
- Estrone - weakly estrogenic
- Must be converted to estradiol
- 17α -hydroxysteroid dehydrogenase (17α -HSD) type 1 is expressed in endometriosis
- In contrast 17α -HSD type 2 inactivates estradiol by catalyzing its conversion to estrone in eutopic endometrial glandular cells during the luteal phase
- Progesterone induces the activity of 17α -HSD
- Inactivation of estradiol to estrone one of the anti-estrogenic properties of progesterone
- 17α -HSD type 2 is absent from endometriotic glandular cells

Mechanisms of Endometriosis

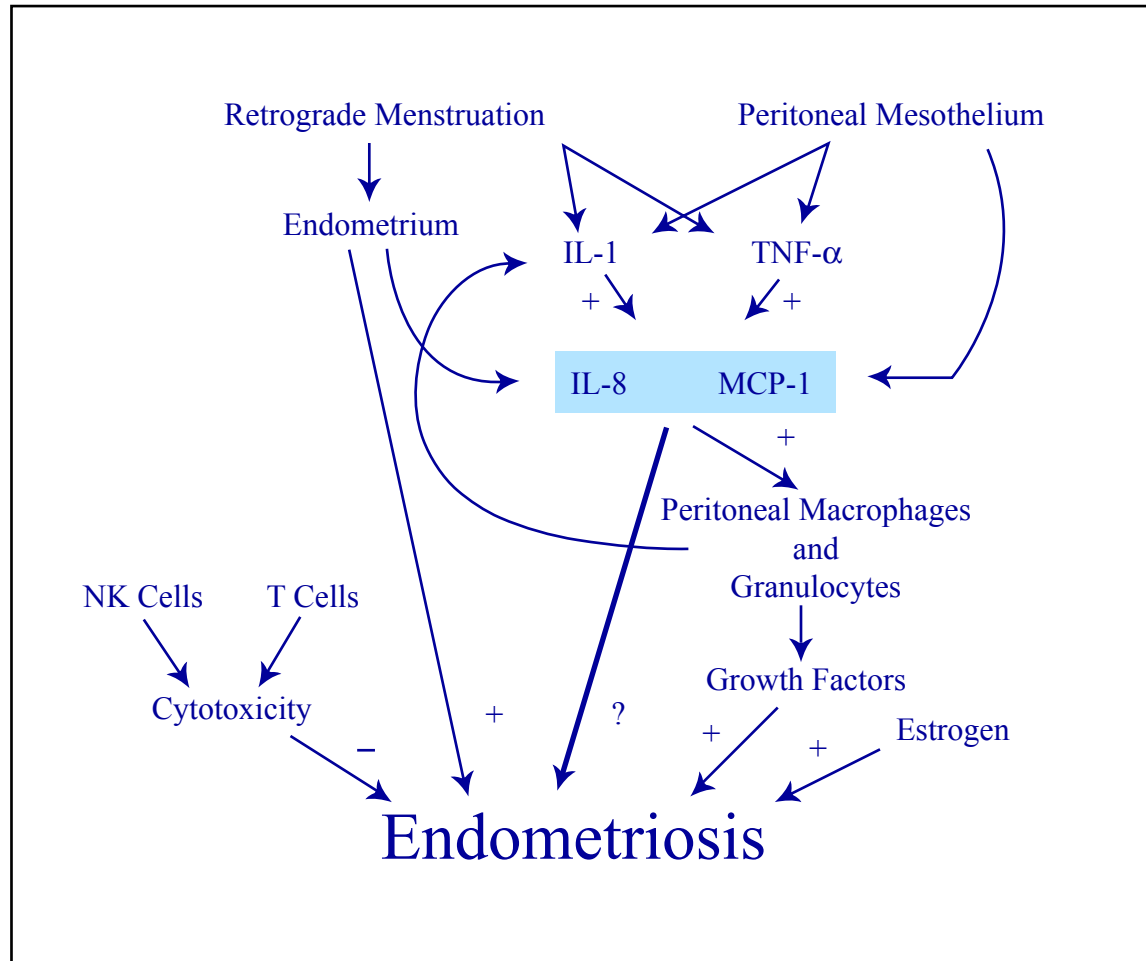


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