Control of the inflammatory response during pregnancy: Why VIP is a Very Important Peptide

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Pregnancy is associated with a maternal-placental immune cooperation status

3 immunological stages

Cellular and molecular mechanisms

- Inflammatory (open wound)
- Anti-inflammatory (fetal growth)
- Inflammatory (delivery)
Pregnancy is associated with a maternal-placental immune cooperation status.

**Cellular and molecular mechanisms**
- Inflammatory (open wound)
- Anti-inflammatory (fetal growth)
- Inflammatory (delivery)

**Pregnancy complications**
- Deep placentation disorders
- Preeclampsia (2-8%)
- IUGR (5-8%)

**Early biomarkers**

After the week 20th
Hypertensive disorders during pregnancy are the second most important cause of maternal death.

In the search of biomarkers of placentation disorders

Cellular and molecular mechanisms

VIP?

anti-inflammatory (fetal growth)

inflammatory (open wound)

inflammatory (delivery)

3 immunological stages
VIP is a pleiotropic polypeptide

- Autonomic nervous system
- Gland secretion
- Vasodilation
- Embryotrophic
- hCG, Progesterone
- Placenta and development
- Anti-inflammatory
- Immunosuppressive

Vasoactive intestinal peptide

28 aa

VIP

Gs/cAMP/PKA

VPAC1/VPAC2 receptors
VIP in short and long loops at the generation and maintenance of the maternal-placental interface

- Trophoblast cell migration/invasion
- Vascular remodelling
- Apoptosis / Phagocytosis
- Control of inflammatory response

Immune homeostasis
- Maternal decidual immune cells (NK, Mø, Treg, DC)

coordinated by trophoblast cells
Potential role of VIP in pregnancy

Does VIP modify trophoblast cell function?

Does it participate in immune-trophoblast interaction?

Is VIP a potential candidate for translational medicine?
VIP increases trophoblast cell migration

Human first trimester trophoblast cell line Swan 71

PKA/CRE signaling is involved in the effect of VIP

PKA/CRE signaling

Cell migration

Fisrt trimester trophoblast cell lines Swan 71 and HTR8

VIP increases trophoblast cell invasiveness

First trimester trophoblast cell line Swan 71

VIP regulates trophoblast cell function

VIP activates Gs, which then activates AC, leading to the production of cAMP. cAMP in turn activates PKA, which can regulate migration and invasion of trophoblastic cells.

VIP receptors VPAC1 and VPAC2 are also involved in this process.

LIF can also promote migration and invasion, possibly through interactions with other pathways or receptors.
VIP induces its own synthesis in Swan trophoblastic cells

Endogenous VIP participates in trophoblast cell migration

VIP silencing

Cell migration

LIF induces VIP and VIP is involved in the effect of LIF.

VIP expression

Cell migration

Potential role of VIP in pregnancy

Does VIP modify trophoblastic cell function?

Does it participate in immune-trophoblast interaction?

Is VIP a potential candidate for translational medicine?
Does VIP participate in the trophoblast-immune interaction?

VIP

VPAC1

VPAC2

Gs

AC

neutrophils

monocytes

macrophages

T lymphocytes

Trophoblastic cells

Other cells at the feto-maternal interface
Macrophages participate in immune homeostasis control at the maternal-placental interface.

Migration of monocytes and differentiation to macrophages

Trophoblast cells

M2 alternative activated macrophages

~ 25\% of leukocytes at the interface

VIP pre-treated trophoblast cells induce an anti-inflammatory profile on monocytes

IL-10

CD39

IL-12

TNF-α
Trophoblastic cells pre-treated with VIP contribute to an M2 activation profile

- **IL-10**: % of CD14+/IL-10+
  - Basal: 20%
  - VIP 100 CM: 35%
  - CM (VIP 100): 40%

- **IL-12**: % of CD14+/IL-12+
  - Basal: 5%
  - VIP 100 CM: 10%
  - CM (VIP 100): 15%

- **CD86**: % of CD14+/CD86+
  - Basal: 10%
  - VIP 100 CM: 15%
  - CM (VIP 100): 20%

- **CD80**: % of CD14+/CD80+
  - Basal: 5%
  - VIP 100 CM: 10%
  - CM (VIP 100): 15%
Trophoblastic cells pre-treated with VIP increase apoptotic cell phagocytosis

Phagocytosis of apoptotic trophoblast cells

monocytes
GM-CSF
5d

macrophages
CM (VIP)
1d

% Phagocytosis of aTb

BASAL
VIP 100
CM
CM (VIP 100)

CD14
CFSE

BASAL 41.9%
VIP 100 48.0%
CM 48.3%
CM (VIP 100) 54.2%
Does trophoblastic VIP modulate monocyte and macrophage function?
VIP-silenced trophoblast cells fail to modulate macrophage function

Lower expression of anti-inflammatory markers

![Graphs showing lower expression of anti-inflammatory markers](image)

Lower phagocytosis of apoptotic cells

![Graphs showing lower phagocytosis of apoptotic cells](image)

VIP in immune-placental interaction
What about neutrophils?

Placenta  Decidua  Myometrium

Monocytes

Macrophages

Mediates LIF effects

Anti-inflammatory and M2 profile
Enhanced apoptotic cell clearance

Spiral artery

Cytotrophoblasts
Syncytiotrophoblasts

Neutrophils
VIP and CM of trophoblast cells inhibit neutrophil extracellular trap (NET) formation.
VIP and CM of trophoblast cells reduce ROS formation in neutrophils

Calo et al, Hum Reprod 2017
VIP and CM of trophoblast cells accelerate neutrophil apoptosis

Calo et al, Hum Reprod 2017
Does endogenous trophoblast VIP participate in neutrophil apoptosis?
CM from VIP-silenced trophoblast cell fail to induce apoptosis in neutrophils

A)

B)
Higher silent phagocytosis of neutrophils induced to apoptosis by trophoblast CM
VIP in immune-placental interaction

Conclusions

VIP?

Monocitos

Increases migration / invasion
Activates autocrine mechanisms
Mediates LIF effects
Induces anti-inflammatory / M2 profiles
Enhances apoptotic cell phagocytosis

Macrófagos

Trophoblastic cells

Deactivates neutrophils
Accelerates apoptosis
Favours their clearance

Neutrophils
VIP in mouse pregnancy models

Experimental mouse models

Switch of immune profile

9 months

Normal pregnancies
- BALB/c x BALB/c
- C57BL/6 x C57BL/6
- CBA x BALB/c

Resorption prone models
- NOD x NOD
- CBA x DBA

VIP Deficient pregnancies
- KO VIP (+/-; -/-)
- KO VPAC1; VPAC2

0.5 - 4.5 - 6.5 - 8.5/9.5 - 12.5 - 19 days

(implantation) (delivery)

Anti-inflammatory predominant profile
VIP in mouse pregnancy models

Implantation sites

VIP in TGC (trophoblast giant cells)

0.5  4.5  6.5  8.5/9.5  19 days (delivery)

Hauk et al, Am J Reprod Immunol 2014
NOD mice pregnancy: VIP treatment improves pregnancy outcome

VIP/PBS injection

0.5  4.5  6.5  8.5/9.5  19 days (delivery)

NOD x NOD mating

Hauk et al, Am J Reprod Immunol 2014
CBA x DBA resorption prone model: VIP treatment improves pregnancy outcome

- Higher number of viable sites
- Higher phagocytosis of apoptotic cells

VIP-deficient pregnancies: Reduced littersize and other parameters altered

<table>
<thead>
<tr>
<th>Mother</th>
<th>Number of pups (P1)</th>
<th>Pregnancy rate</th>
<th>Implantation sites distribution</th>
<th>Period between gestations (days)</th>
<th>Number of pups with weight ≤1,3g</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>8.3 ± 0.5</td>
<td>17/20</td>
<td>Symmetric</td>
<td>22.5 ± 1.5</td>
<td>3/10</td>
</tr>
<tr>
<td>+/-</td>
<td>5.8 ± 0.8</td>
<td>9/14</td>
<td>Asymmetric</td>
<td>40.5 ± 5.25</td>
<td>7/14</td>
</tr>
<tr>
<td>+/-</td>
<td>2.9 ± 1.4</td>
<td>5/16</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2/4</td>
</tr>
</tbody>
</table>
VIP (+/-) Trophoblast Giant Cells (TGC) monolayer in implantation sites is altered (wider and disorganized)

Implantation sites

WT trophoblast cells (TGC VIP +/-)

(VIP +/-) trophoblast cells (TGC VIP +/-)

Hauk et al, poster
VIP (+/-) trophoblast cells display lower outgrowth migration in vitro

Reduced trophoblast cell migration of ectoplacental cones (EPC)

WT females
VIP (-/-) or WT males

0,5 4,5 7,5/8,5 (implant) 19 gestation days (delivery)

WT
VIP +/-
VIP is involved in autocrine loops for ectoplacental cone trophoblast cell invasiveness.
Lower expression of MMP9 in VIP (+/-) trophoblast giant cells (TGC)

Laser Capture Microdissection

Lower expression of MMP9 mRNA levels in VIP deficient TGC
Lower expression of anti-inflammatory markers in sites with VIP deficient trophoblast cells

Immunological markers in implantation sites

TGC (VIP +/-)
Pregnancies with VIP deficient trophoblast cells: Reduced fetal weight and higher resorption rates

- Females: WT or VIP (-/-)
- Males: WT

Timeline:
- 0.5 - 4.5 - 7.5 - 14.5 - 19 gestation days
- (implant) - (delivery)

Graphs:
- Fetal weight (g)
- Placental weight (g)
- Reabsorption rate

Hauk et al, poster
VIP in pregnancy

Does VIP modify trophoblastic cell function?

VIP increases human trophoblast cell migration and invasion through autocrine loops. Similar results observed in murine pregnancy

Does it participate in immune-trophoblast interaction?

VIP regulates immune-trophoblast interaction that would favor immune homeostasis through autocrine and paracrine loops

- Promotes M2 activated macrophages
- Enhances apoptotic cell silent clearance
- Deactivates neutrophils and accelerates their apoptosis
- Promotes an immunosuppressant microenvironment at the interface

Is VIP a candidate for translational medicine?
our current views and questions

VIP in human pregnancy

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Thanks
Global distribution of Maternal Mortality Ratio (MMR)

Maternal Mortality Ratio per 100,000 live births in 2015

In 2000, leaders of 189 countries signed a declaration at UN on 8 Millennium Development Goals (MDG) in public health

**MDG 5:**
calls for a 75% reduction in MMR between 1990 and 2015

Achievement 2015: 43.9%

Source: Based on WHO 2015a; map re-created based on WHO 2015a.
VIP is expressed in human trophoblast cells of first trimester placental columns

Modified from Ferretti et al Human Reprod Update 2007

Paparini D, Submitted abstract to IFPA 2017 in collaboration with Prof. John Aplin, Univ. Manchester, UK
Lower recruitment of Treg cells to implantation sites with VIP deficient trophoblast cells

Lower number of Treg cells in implantation sites

females males
Foxp3 GFP WT VIP (-/-) or WT
Lower expression of vascular remodeling markers in sites with VIP deficient trophoblast cells

females
WT
males
VIP (-/-) or WT

0,5  4,5  8,5                      19
(implant)                        (delivery)

Lower expression of vascular remodeling markers

Angpt1

Relative mRNA levels

WTxWT  WTxKO

Vegfa

Relative mRNA levels

WTxWT  WTxKO

n=6  n=6

*  **
VIP receptor antagonist reduces ectoplacental cone trophoblast cell invasiveness

Lower invasiveness of ectoplacental cone trophoblast cells in the presence of a VIP receptor antagonist

Invasiveness of trophoblast cells in vitro
Trophoblastic cells pre-treated with VIP increase monocyte migration

Monocyte migration

Chemokine expression in trophoblast cells
Apoptotic cell phagocytosis: Phagocytic synapsis formation

‘eat me’ signals and receptors

Apoptotic cell

Phagocytic cell

PS (phosphatidyl serine)

TSP1 (thrombospondin1)

Gas6, MFG-E8

αvβ3 integrin

PSR, TAM, others

BAI1, TIM4, stabilin-2, RAGE

EMBO Mol Med (2013) 5, 661–674
CM(VIP) – induced apoptotic cell phagocytosis involves TSP-1 and αvβ3 integrin

Expression of αvβ3 in CD14+ cells

CD14+ αvβ3+ cells

CM (VIP) + apoptotic cells
VIP pre-treated trophoblast cells enhance apoptotic cell phagocytosis.