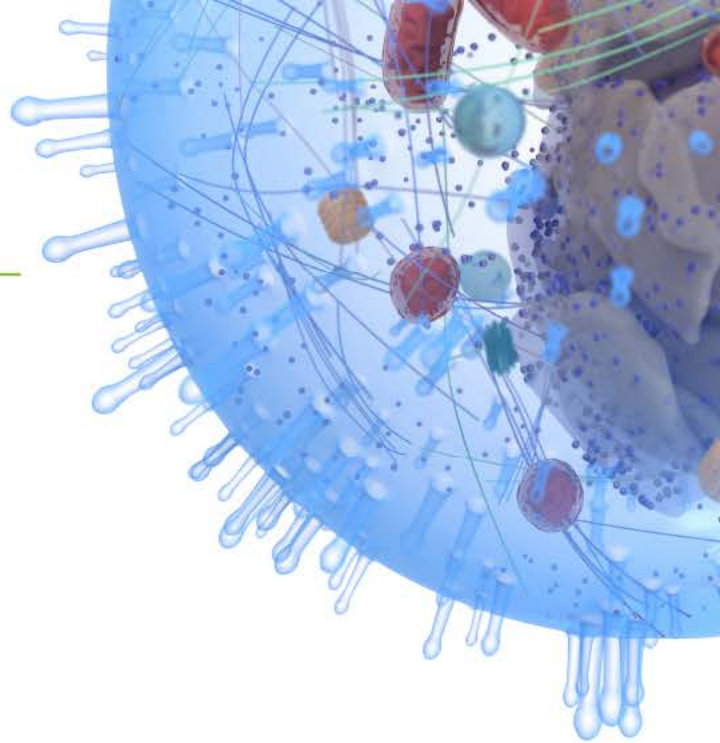
ICoMI VIRTUAL
2nd International Congress of
Micro-immunotherapy
June 2-4, 2022



PIF / sPIF

From Nature to Clinics

Effective Embryo-Maternal Interaction. Clinical Management to Achieve Immune Homeostasis.

Eytan R. BARNEA, MD, FACOG
BioIncept™, LLC (PIF* Proprietary)

Elected Member (Assoc)
Childbirth and Postpartum Hemorrhage Committee of the
F.I.G.O. International Federation of Gynecologists and Obstetricians
Founder and Chairman
S.I.E.P The Society for the Investigation of Early Pregnancy

Ask not what you can do for the embryo…

PIF SCIENCE

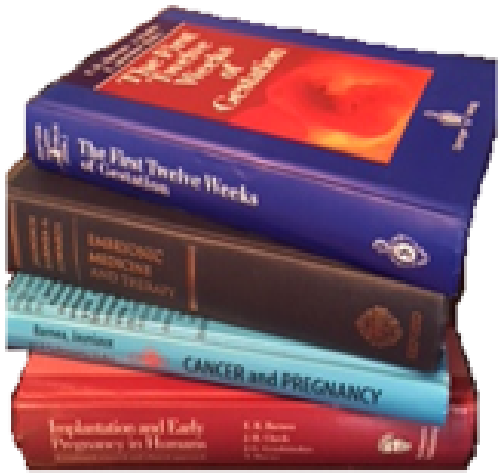
Retrieve Pregnancy's Immunity & Transplant Regulation
Transpose PIF Effects in Pregnancy and/or Non-Pregnant Applications

Ask what the embryo can do for YOU!

TM

EMPHASIS ON EARLY PREGNANCY INVESTIGATION

Promote Fundamental Concepts (i.e. PIF)



The First Twelve Weeks of Gestation
Barnea, E.R., *Hustin, J., Jauniaux, E. (eds.) (Berlin: Springer-Verlag) 1992*

Implantation and Early Pregnancy in Humans
Barnea, E.R., *Check, J.H., Grudzinskas, J.G., Maruo, T., (eds.). (Carnforth: Parthenon Publishing) 1994*

Embryonic Medicine and Therapy
Jauniaux, E., ***Barnea, E.R.****, Edwards, R.G. (eds.) (Oxford: Oxford University Press) 1997*

Cancer and Pregnancy
Barnea, E.R*. Jauniaux. E., Schwartz, P.E. (eds.). (London: Springer) 2001*

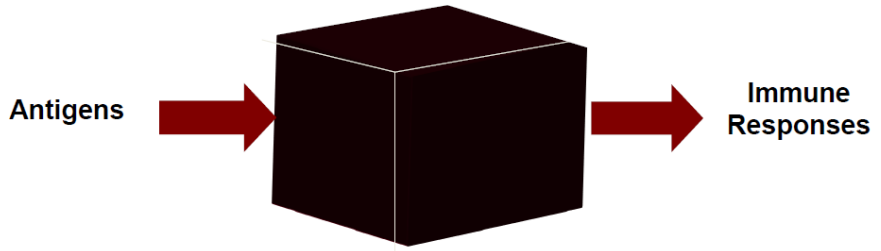
Trophoblast Research V15
Miller, R., ***Barnea, E.R.****, Myatt, L. (eds.) (London: W.B. Saunders) 2001*

TM

EVOLVING IMMMUNOTHERAPY APPROACH

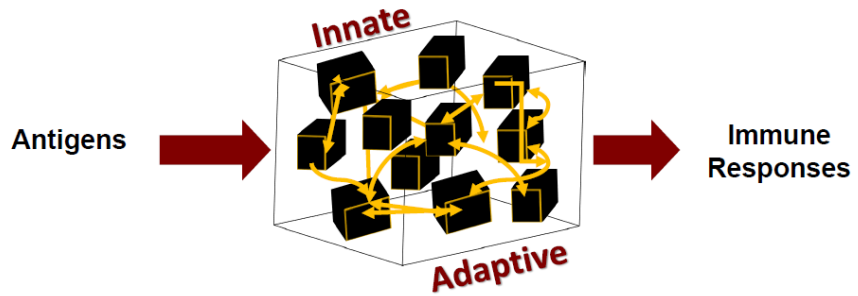
PIF Adapted to Need

Previous



Generalized immunosuppression associated with risk of infection and malignancy

Current



Targets specific pathways using combined immunosuppression to lower risks of infection and malignancy

TM

PIF - The Future

Integrated local & systemic Immune modulation. Selective target and pathway involved

sPIF FIRST-IN-HUMAN CLINICAL PHASE 1 TRIAL IMMUNE DISORDER
Completed Successfully; Enables Progress To Phase 2

PIF AIH Phase 1 SAD Clinical Trial
STATUS COMPLETE

- 23 patients screened
- 18 patients successfully enrolled
- All 18 patients completed the trial
- No side effects by H&P or lab results
- Pharmacokinetics: rapid PIF clearance

PIF AIH Phase 1 MAD
STATUS COMPLETE

- 19 patients screened
- 18 patients successfully enrolled
- All 18 patients completed the trial, no dropouts
- No side effects by H&P or lab. results
- No anti-PIF antibody

Randomized, Double-Blind, Placebo-Controlled Study

- First-in-Human study.
  AIH: representative complex immune disorder
- FDA: Orphan Drug Designation
- **SAD** PIF Rx (0.1, 0.5, 1mg/kg one injection)
- **MAD** PIF Rx (0.1, 0.5, 1mg/kg for 5 consecutive days)
- No volunteers required by FDA
- Patients on multiple drug regimen
- www.ClinicalTrials.gov

www.clinicaltrials.gov

- **sPIF: no toxicity**
- **sPIF: no deleterious drug-to-drug interaction**
- **sPIF: no anti-PIF antibody develops**
- **sPIF: rapid clearance / long term effect**
- **sPIF: exploratory efficacy**

FDA IMPARTS sPIF PRIVILEGED STATUS

Orphan Drug Designations (ODD) & FAST-TRACK Award

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

FAST-TRACK

IND 119219

GRANT FAST TRACK

Christopher O'Brien, M.D., A.G.A.F., F.R.C.M.I.
Chief of Clinical Hepatology
Center for Liver Diseases
1500 NW 12 Avenue
Suite 1101
Miami, FL 33136

Dear Dr. O'Brien:

Please refer to your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act for synthetic PreImplantation Factor (sPIF).

We also refer to your July 18, 2013, request for Fast Track designation. We have reviewed your request and concluded that it meets the criteria for the Fast Track designation. Therefore, we are designating as a Fast Track development program the investigation of synthetic PreImplantation Factor (sPIF) for the treatment of autoimmune hepatitis. Please note that if the clinical development program you pursue does not continue to meet the criteria for Fast Track designation, the application will not be reviewed under the Fast Track program.

For further information regarding Fast Track Drug Development Programs, please refer to the FDA document "Guidance for Industry on Fast Track Drug Development Programs: Designation, Development, and Application Review". This document is available on the internet at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079736.pdf or may be requested from the Office of Communications, Division of Drug Information at 301-796-3400 or 1-888-463-6332.

If you have any questions, contact CDR Anissa Davis-Williams, Regulatory Project Manager, at (301) 796-5016.

FDA U.S. FOOD & DRUG ADMINISTRATION

Office of Orphan Products Development
Food and Drug Administration
WO32- 5295
10903 New Hampshire Avenue
Silver Spring, MD 20993

NOV 2 2 2017

ODD in AIH

BioIncept, LLC
140 E 40th St
New York, N

Attention:

Re: Designa
Date
Recei

Dear Dr. Bar

This letter re
for "treatmer

FDA U.S. FOOD & DRUG ADMINISTRATION

Office of Orphan Products Development
Food and Drug Administration
WO32- 5295
10903 New Hampshire Avenue
Silver Spring, MD 20993

MAY 0 8 2018

ODD in GvHD

BioIncept, LLC
140 E. 40th Street, #11E
New York, NY 10016

Attention: Eytan R. Barn
Chief Scientifi
Eytan.Barnea@

Re: Designation request # DI
Amendment Dated:
Amendment Received

Dear Dr. Barnea:

This letter responds to your a
preimplantation factor for "pr

FDA U.S. FOOD & DRUG ADMINISTRATION

Office of Orphan Products Development
Food and Drug Administration
WO32- 5295
10903 New Hampshire Avenue
Silver Spring, MD 20993

DEC 1 9 2018

ODD in ARS

BioIncept, LLC
140 E 40th Street, #11E
New York, NY 10016

Attention: Eytan R. Barnea, MD, FACOG
Founder & Chief Scientist
Eytan.Barnea@BioIncept.com

Re: Designation request # DRU-2018-6649
Dated: October 11, 2018
Received: October 15, 2018

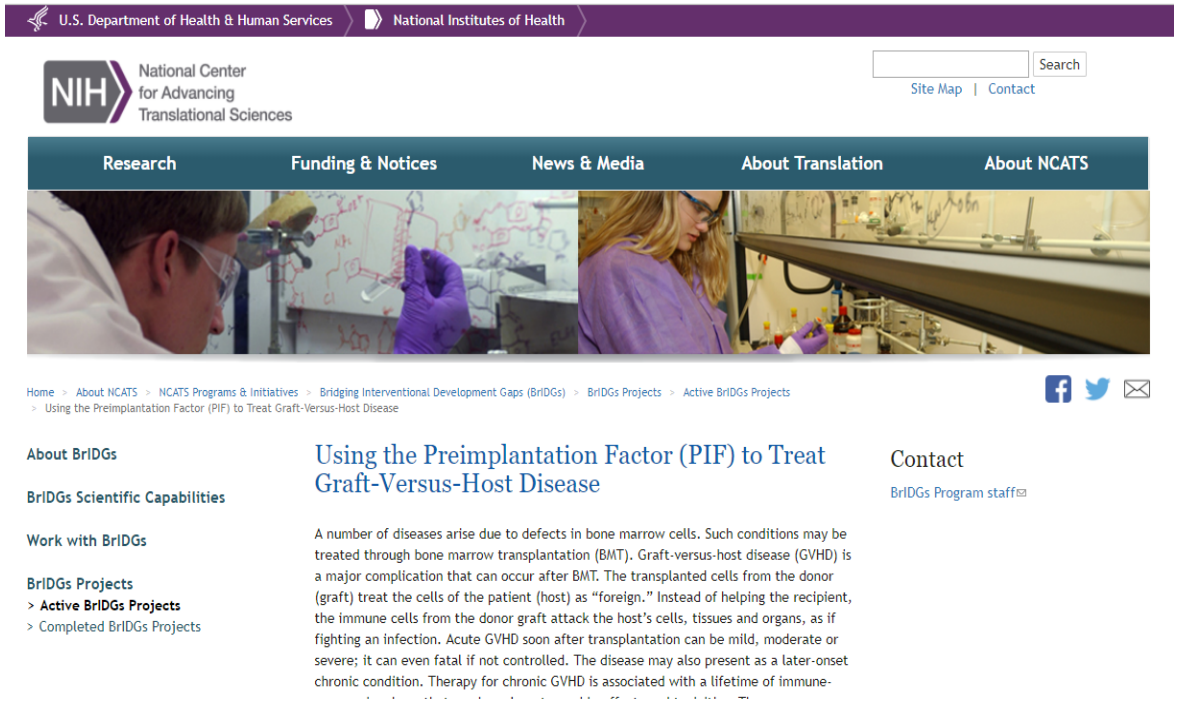
Dear Dr. Barnea:

This letter responds to your request for orphan-drug designation of synthetic preimplantation factor for "treatment of acute radiation syndrome."

TM

NIH / NCATs BrIDGs SUPPORTS sPIF DEVELOPMENT TO IND

Long-Term FDA Mandated Toxicity Studies (3,6 & 9months)



BrIDGs program assists in advancing promising therapeutic agents through late-stage pre-clinical development toward an Investigational New Drug (IND) application and clinical testing

BrIDGs: sPIF / Chronic GVHD

- NIH/NCATS Drug to Market Plan
- Support includes: 3m tox successfully completed,
- Long-Term Toxicology 6mo murine and 9mo canine is advanced as required by FDA

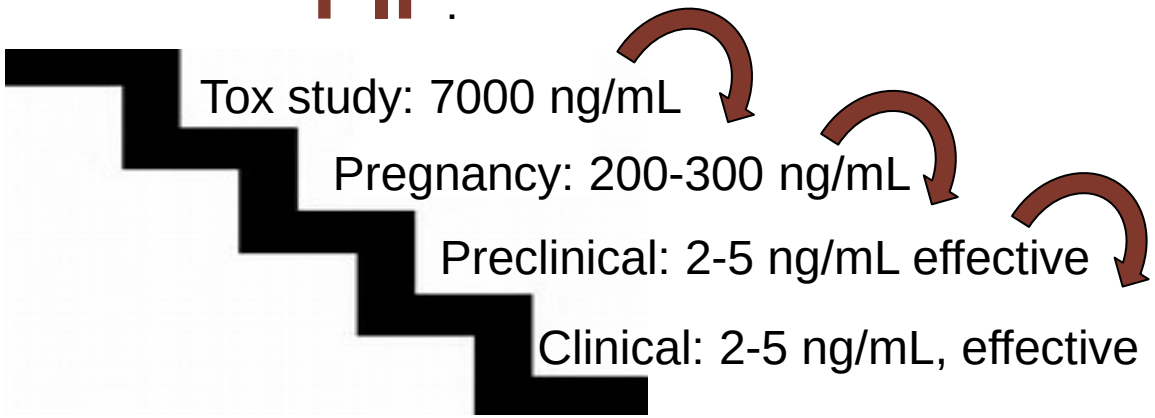
TM

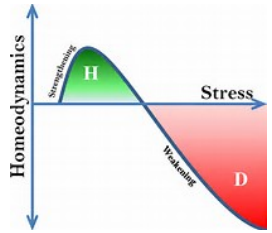
PIF NATURE-BASED MONOIMMUNE THERAPY

MicroImmunotherapy (Cytokine cocktail in Mircodoses):

vs.

PIF:





http://sureshrattan.com/category/researchs/c37-hormesis/

Hormesis

High doses are nontoxic and show rapid clearance

TM

Short term, low dose **PIF** → long term therapeutic effects
Modulates Th1/Th2, TH-17 response

STEROIDS / IMMUNOSUPPRESSANTS vs PIF

High Safety & Devoid of Side-Effects

Fever, weight loss; extreme tiredness; diarrhea; stomach pain; warm, red, or painful skin; painful, difficult, or frequent urination; other infections. tuberculosis or hepatitis B infection, severe or life-threatening cancers including lymphoma. hepatosplenic T-cell lymphoma (HSTCL), that often causes death within a short period of time. Increased blood pressure, weight gain, High blood sugar, Osteoporosis/bone fractures poor wound healing

sPIF

FDA DIRECTED TOXICOLOGY
NON-TOXIC
NO SIDE EFFECTS
Completed high dose long term
SQ sPIF daily administration
(mice/dogs)
Rapid clearance

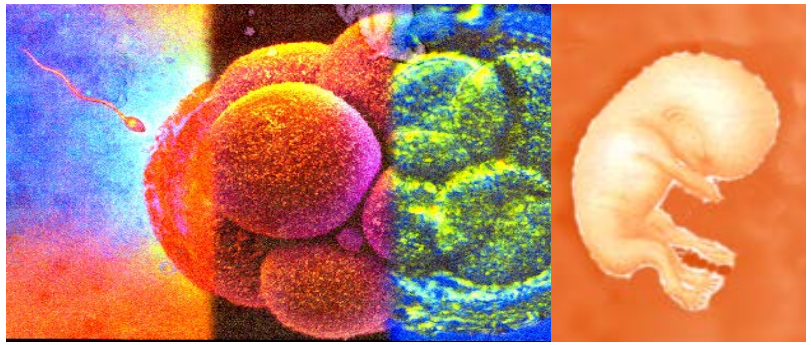
Clinical Grade sPIF

sPIF Lot 0127-13106-3
Produced at PolyPeptide
Bottled/Sterilized at
ColdStream

PIF: PREIMPLANTATION FACTOR - ESSENTIAL FUNCTION

Immune Regulation & Transplant Acceptance

PIF - EMBRYOGENESIS

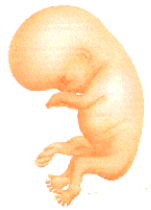


Embryo / Allograft Immune Tolerance & Acceptance
without Maternal / Host Immune Suppression

PIF ESSENTIAL for MATERNAL RECOGNITION

Early Signal from Embryo / Allograft to Mother /Host from 2cell - Throughout Pregnancy

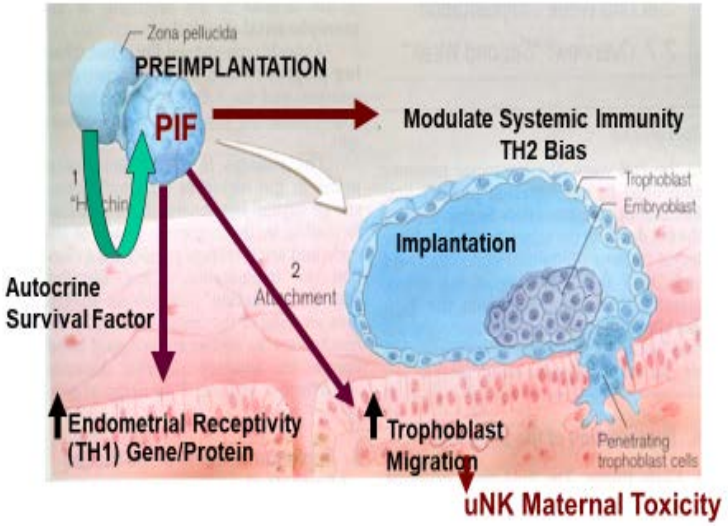
NON-VIABLE EMBRYO
DOES NOT PRODUCE **PIF**
REJECTED BY MOTHER/HOST
IMMUNE INTOLERANCE

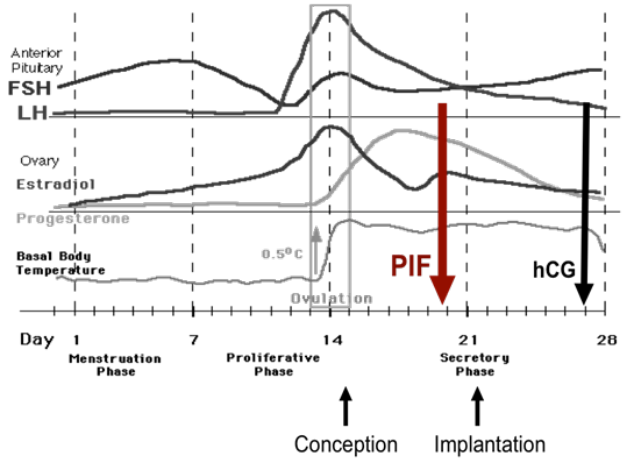


VIABLE EMBRYO
PRODUCES **PIF**
ACCEPTED BY MOTHER/HOST
IMMUNE MODULATION w/o SUPPRESSION

PIF – PREGNANCY SPECIFIC EMBRYO SIGNAL

Role and Action from Fertilization until Delivery





***** **hCG** is detected 5-7 days **POST** implantation

PIF: FIRST SIGNAL

- Secreted at 2-cell embryo
- Promotes maternal recognition / embryo acceptance
- No PIF No pregnancy

PreImplantation PIF:

- Trophic / protective effect on embryo
- Initiate systemic maternal immune tolerance
- Prime locally endometrium

PIF at implantation: immune acceptance

- Enhance endometrial receptivity at /after implantation
- Regulate controlled trophoblast invasion
- Promote trophoblast tolerance (HLA-G/progesterone)

PIF during fetal period: maintain and protect

- Protect against fetal loss
- Reduce systemic inflammation
- Increase binding to adaptive immunity, Th2/Th1 cytokine bias

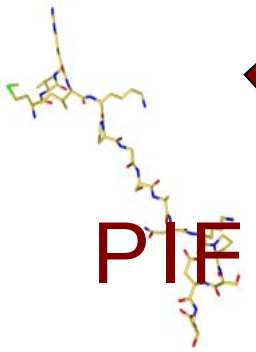
PIF at term / delivery: tolerance signal withdrawn

- Placental PIF expression is minimal
- Circulating levels low

PIF: NOT detected post-pregnancy

PIF: ESSENTIAL INTEGRATED ROLE THROUGHOUT PREGNANCY

Embryo / Endometrium / Trophoblast / Immune System



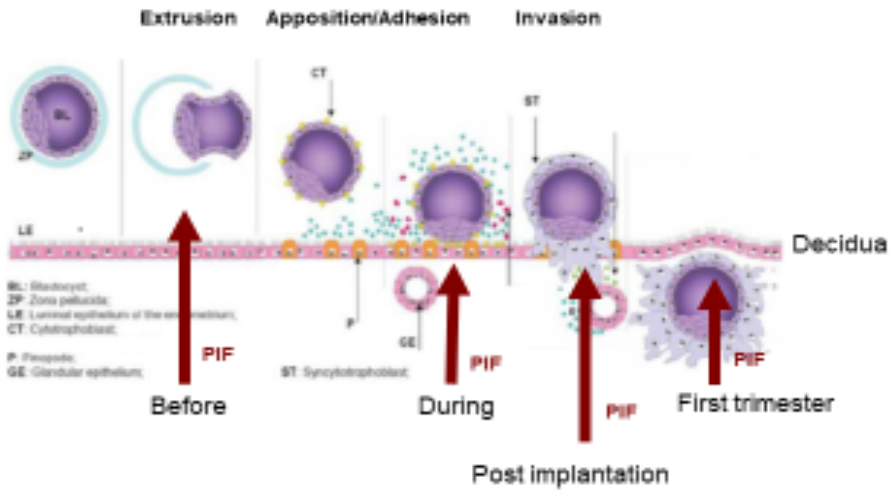
PIF

secreted & targets the embryo	Promote & Protect Embryo
culture media & maternal serum	Detect Embryo Viability Pregnancy Biomarker
regulates maternal immunity	Immune Homeostasis without Suppression
acts on endometrium	Enhance Endometrial Receptivity
acts on trophoblast	Improve Placental Acceptance
Placental / systemic effects	Prevent Fetal Loss spontaneous and LPS induced

TM

sPIF PROMOTES EMBRYO IMPLANTATION

Creates a Favorable Endometrial Environment



Implantation is still considered a "black box". Viable embryo and endometrial receptivity are a must for reproductive success.

- **sPIF** primes the endometrium – pre -, during and post - implantation
- **sPIF** promotes endometrial receptivity / controlled trophoblast invasion
- **sPIF** promotes favorable uterine environment to prevent embryo loss.

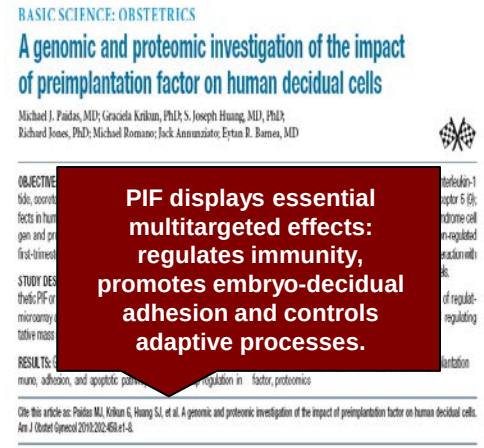
RB&E

AJOG

Fitzgerald, 2008 adapted

sPIF ROLE: PRIME / SUPPORT / PROTECT

Promotes Endometrial Receptivity

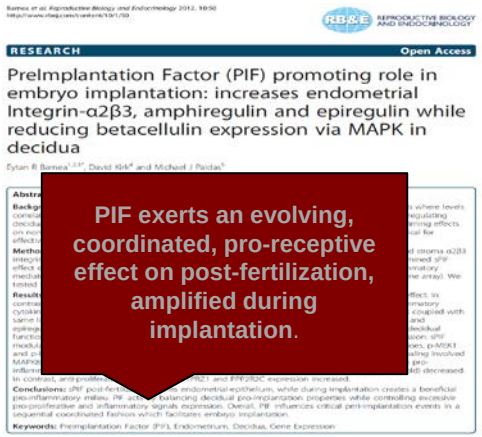


BASIC SCIENCE: OBSTETRICS

A genomic and proteomic investigation of the impact of preimplantation factor on human decidual cells

Michael J. Paidas, MD; Graciela Krikun, PhD; S. Joseph Huang, MD, PhD; Richard Jones, PhD; Michael Romano; Jack Annunziato; Eytan R. Barnea, MD

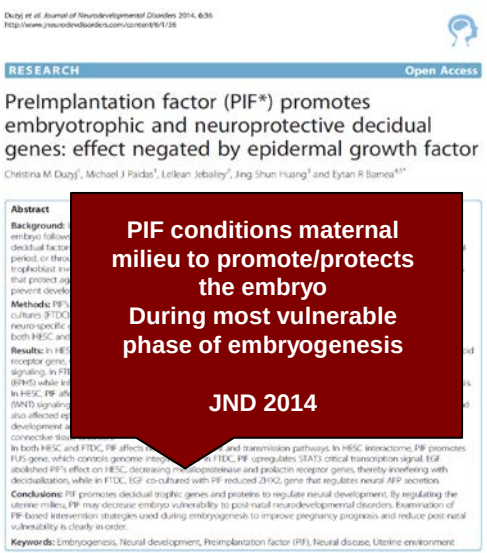
PIF displays essential multitargeted effects: regulates immunity, promotes embryo-decidual adhesion and controls adaptive processes.



RESEARCH Open Access

Preimplantation Factor (PIF) promoting role in embryo implantation: increases endometrial Integrin-α2β3, amphiregulin and epiregulin while reducing betacellulin expression via MAPK in decidua

PIF exerts an evolving, coordinated, pro-receptive effect on post-fertilization, amplified during implantation.

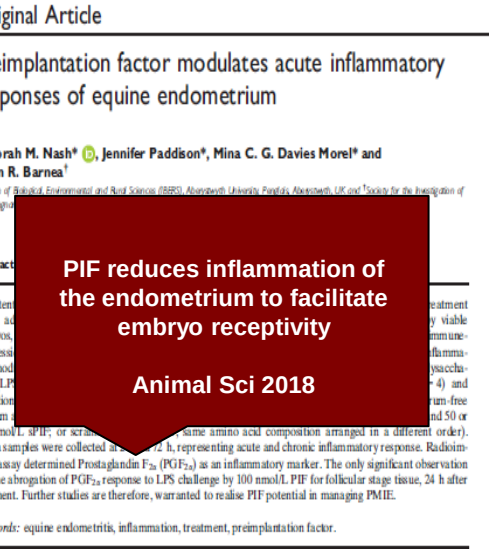


RESEARCH Open Access

Preimplantation factor (PIF*) promotes embryotrophic and neuroprotective decidual genes: effect negated by epidermal growth factor

PIF conditions maternal milieu to promote/protects the embryo
During most vulnerable phase of embryogenesis

JND 2014



Original Article

Preimplantation factor modulates acute inflammatory responses of equine endometrium

Deborah M. Nash*, Jennifer Paddison*, Mina C. G. Davies Morel* and Eytan R. Barnea†

PIF reduces inflammation of the endometrium to facilitate embryo receptivity

Animal Sci 2018

PIF PROMOTES TOLERANCE / ACCEPTANCE

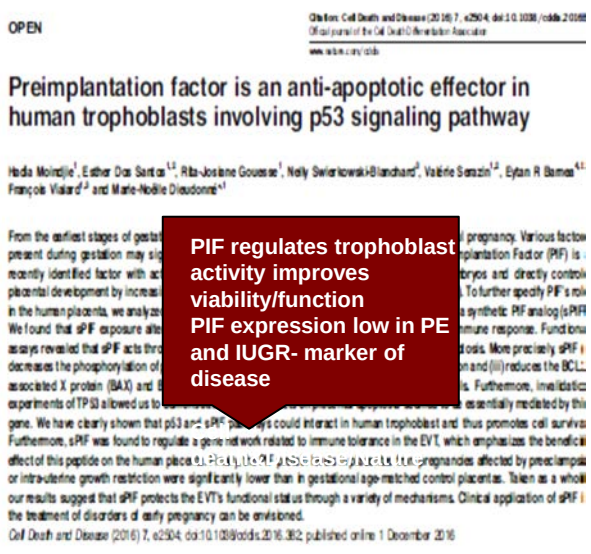
Regulates Trophoblast Invasion

SMFM PAPERS www.AJOG.org

Preimplantation factor promotes first trimester trophoblast invasion

Christina M. Duzyj, MD, MPH; Eytan R. Barnea, MD; Min Li, PhD; S. Joseph Huang, MD, PhD; Graciela Krikun, PhD; Michael L. Paidas, MD

PIF shows potential preventative or therapeutic role for pregnancy complications associated with inadequate trophoblast invasion.



OPEN

Preimplantation factor is an anti-apoptotic effector in human trophoblasts involving p53 signaling pathway

Hadia Moindjie, Esther Dos Santos, Rita-Josiane Gouesse, Nelly Swierkowski-Blanchard, Valérie Serazin, Eytan R Barnea, François Vialard and Marie-Noëlle Dieudonné

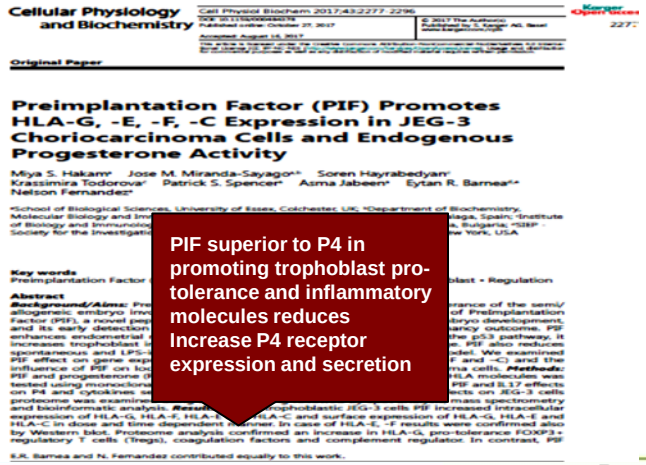
PIF regulates trophoblast activity improves viability/function
PIF expression low in PE and IUGR- marker of disease

BIOLOGY OF REPRODUCTION (2014) 91(5):118, 1–10

Preimplantation Factor (PIF) Promotes Human Trophoblast Invasion

Hadia Moindjie, Esther Dos Santos, Laurence Loeuillet, Héloise Gronier, Philippe de Mazancourt, Eytan R. Barnea, François Vialard, and Marie-Noëlle Dieudonne

PIF directly controls human trophoblast invasion; PIF is involved in pathological pregnancies where trophoblast invasion is insufficient or excessive



Cellular Physiology and Biochemistry

Original Paper

Preimplantation Factor (PIF) Promotes HLA-G, -E, -F, -C Expression in JEG-3 Choriocarcinoma Cells and Endogenous Progesterone Activity

Miya S. Hakam, Jose M. Miranda-Sayago, Soren Hayrabedyan, Krassimira Todorova, Patrick S. Spencer, Asma Jabeen, Eytan R. Barnea, Nelson Fernandez

PIF superior to P4 in promoting trophoblast pro-tolerance and inflammatory molecules reduces
Increase P4 receptor expression and secretion

sPIF INTEGRATED LOCAL & SYSTEMIC PROTECTIVE EFFECT

Reduces At-Risk Pregnancy

Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy

Roumen G Roussev [a], Boris V Dons'koi [b], Christopher Stamatkir Sivakumar Ramu [a], Viktor P Chernyshov [b], Carolyn B Coulam [a], Eytan R Barnea [c,d,e,*]

[a] CARI Reproductive Institute/BioIncept LLC, Chicago, IL, United States; [b] Laboratory of Immunology, In Obstetrics and Gynecology, Academy of Medical Sciences, Kiev, Ukraine; [c] SIEP – Society for the Inves Pregnancy, Cherry Hill, NJ, United States; [d] BioIncept LLC, Cherry Hill, NJ, United States; [e] Departme of Obstetrics, Gynecology and Reproduction, UMDNJ – Robert Wood Johnson Medical School, Camden,
* Corresponding author. E-mail address: barnea@earlypregnancy.org (ER Barnea).

Dr Eytan R Barnea, MD, FACOG is double board certified in obstetrics, gynaeco endocrinology. He investigates embryo-derived signalling in pregnancy, translating clinical applications in pregnancy and immune disorders. He is the founder of the Society Early Pregnancy, director of obstetrics and gynaecology at CAMcare and associate clinica and gynaecology and reproduction at University of Medicine and Dentistry of New Jerse Medical School. In n Medicine Ministry/Jewish Na

Abstract Embryo-secreted preimplantation orrelates v ment in humans by promoting implantation ar systemic tive in autoimmune disease models. sPIF b g to immu suppression. This study examined the effect of sPIF on natu killer (NK) cell cytotoxicity in 107 consecutive nant patients with recurrent pregnancy loss (RPL) and 26 infertile IVF patients (controls). The effects of sPI immunoglobulin (Ig), Intralipid and scrambled PIF (PIFscr; negative control) on NK cell cytotoxicity to peripl compared by flow cytometry of labelled-K562 cell cytolysis. The effects of sPIF and PIFscr on whole-blood NK also compared. In patients with RPL, sPIF inhibited NK cell cytotoxicity at doses of 2.5 and 25 ng/ml (37% an PIFscr (18%; $P < 0.001$), regardless of the proportion of peripheral-blood NKCD56+ cells to lymphocytes. Pr from infertile patients with sPIF for 24 h decreased NKCD69+ expression versus incubatino with PIFscr ($P < 0.0$ inhibits NK cell cytotoxicity by reducing NKCD69 expression, suggesting a significant role in RPL patients.

PIF reduces circulating NK cytotoxicity In RPL patients RBMO 2013

RESEARCH ARTICLE

Synthetic PreImplantation Factor (PIF) prevents fetal loss by modulating LPS induced inflammatory response

Nicoletta Di Simone[1☯], Fiorella Di Nicuolo[1,2☯], Riccardo Marana[1,2], Roberta Castellani[1], Francesco Ria[3], Manuela Veglia[1], Giovanni Scambia[1], Daniel Surbek[4], Eytan Barnea[5,6¤], Martin Mueller[4,7¤*]

1 Department of Obstetrics and Gynecology, Università Cattolica Del Sacro Cuore, A. Gemelli Universitary Hospital, Rome, Italy, 2 International Scientific Institute Paolo VI, ISI, Università Cattolica Del Sacro Cuore, A. Gemelli Universitary Hospital, Rome, Italy, 3 Institute of General Pathology, Università Cattolica Del Sacro Cuore, Rome, Italy, 4 Department of Obstetrics and Gynecology, University Hospital Bern, Bern, Switzerland, 5 The Society for the Investigation of Early Pregnancy (SIEP), Cherry Hill, New Jersey, United States of America, 6 BioInoept LLC, Cherry Hill, New Jersey, United States of America, 7 Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut, United States of America

☯ These authors contributed equally to this work.
¤ These authors also contributed equally to this work.
* martin.mueller@insel.ch

Abstract

Maternal control of inflammation is essential during pregnancy and an exaggerated response is on y response is mediated by multiple fact ation of TLRs results in NALP-3 media otein containing a CARD (ASC) and cas o-inflammatory cytokines IL-1β and IL-18 nvestigated PreImplanta-tion Factor (PIF tion in pregnancy. Addi-tionally, synthe e disorders. We used a LPS induced murine mode fetal loss and synthetic PIF reduced this fetal loss and increased the embryo weight significantly. We detected increased PIF expression in the placentae after LPS insult. The LPS induced serum and placenta cytokines were abolished by synthetic PIF treatment and importantly synthetic PIF modulated key members of inflammasome complex NALP-3, ASC, and caspase-1 as well. In conclusion our results indicate that synthetic PIF protects against LPS induced fetal loss, likely through modulation of inflammatory response especially the inflammasome complex. Given that synthetic PIF is currently tested in autoimmune diseases of non-pregnant subjects (clinicaltrials.gov, NCT02239562), therapeutic approach during pregnancy can be envisioned.

PIF has preventative or therapeutic role for reducing pregnancy complications. PLOS One

Journal of Reproductive Immunology 114 (2016) 58–64

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journal homepage: www.elsevier.com/locate/jreprimm

PreImplantation Factor (PIF*) endogenously prevents preeclampsia: Promotes trophoblast invasion and reduces oxidative stress

E.R. Barnea[a,b,*], F. Vialard[c,d], H. Moindjie[d], S. Ornaghi[e,f], M.N. Dieudonne[c], M.J. Paidas[f]

[a] Society for the Investigation of Early Pregnancy, 1697 Lark Lane, Cherry Hill, NJ 08003, USA
[b] BioIncept, LLC, 1697 Lark Lane, Cherry Hill, NJ 08003, USA
[c] UPRES-EA 2493, Université de Versailles-St-Quentin, Unité de Formation et de Recherche des Sciences de la Santé-Simone Veil, Montigny-le-Bretonneux, France
[d] Department of Biology of Reproduction, Cytogenetic, Gynecology and Obstetrics, Centre Hospitalier de Poissy-Saint Germain, 23 Boulevard Gambetta, Poissy, France
[e] Department of Obstetrics and Gynecology, University of Milano-Bicocca, Piazza dell'Ateneo Nuovo, 1, Milano, Italy
[f] Department of Obstetrics, Gynecology and Reproductive Sciences, Yale Women and Children's Center For Blood Disorders and Preeclampsia Advancement, Yale University School of Medicine, 333 Cedar Street, FMB 3398, New Haven, CT 06520-8063, USA

ARTICLE

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Preeclampsia prevention
Trophoblast invasion
Embryo protection

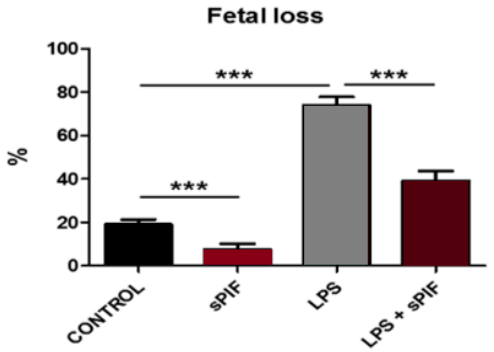
se patho-physiology is initiated early in gestation, while late pregnancy. Thus, prevention should optimally be tion between PIF, secreted early by viable embryos, and echanisms of preeclampsia prevention.

underlying preeclampsia. At first, shallow implantation protein misfolding, and endothelial dysfunction. Later of maternally derived oxygenated blood compromises the placenta. The first is likely involved in early preeclampsia occurrence due to reduced effectiveness of trophoblast/uterus interaction. The latter is observed with later-onset preeclampsia, caused by a breakdown in placental blood flow regulation. We reported that 1. PIF promotes implantation, endometrium receptivity, trophoblast invasion and increases pro-tolerance trophoblastic HLA-G expression and, 2. PIF protects against oxidative stress and protein misfolding, interacting with specific targets in embryo, 3. PIF regulates systemic immunity to reduce oxidative stress. Using PIF as an early preventative preeclampsia intervention could ameliorate or even prevent the disease, whose current main solution is early delivery.

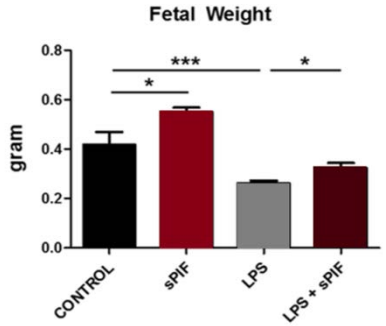
Reduced placental PIF expression associated with Preeclampsia JRI 2015

sPIF REDUCES FETAL LOSS- INTACT IMMUNE SYSTEM

Spontaneous ~3Fold & Inflammation-Induced ~2Fold Decrease (murine)



sPIF 1mg/kg continuous treatment from conception to Day 14, n=18/group, (***<0.001)



sPIF improves fetal weight

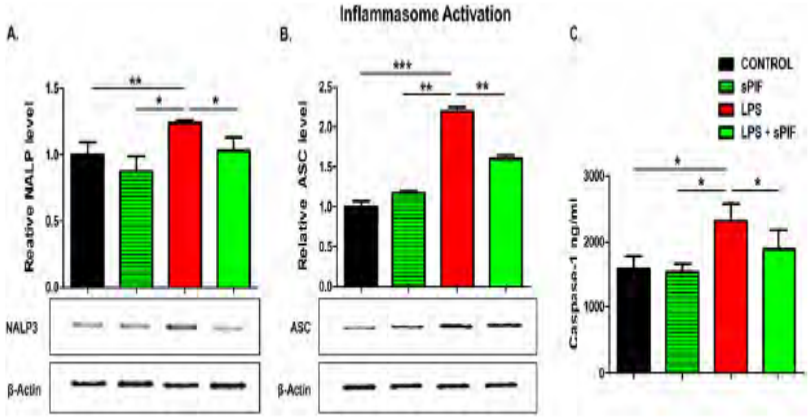


Fig 3. Inflammasome pathway analysis (18±20 placental lysates for each group). Representative gel images of **(A)** relative NALP-3 and **(B)** relative ASC protein levels in placental tissue lysates. The downstream cytokine caspase-1 levels in lysates were measured using enzyme-linked immunoassay **(C)**. Data are mean ± SD. β-Actin was the control. *p<0.05. sPIF: synthetic PreImplantation Factor; LPS: Lipopolysaccharides.

PLOS ONE | Synthetic PreImplantation Factor (PIF) Prevents Fetal Loss by Modulating LPS Induced Inflammatory Response

Spontaneous miscarriage occurs in 15% of all pregnancies.
Bacteria / Inflammation cause fetal death

Preventing miscarriage of viable embryos is challenging

- **sPIF** reduces miscarriage rate in preserved maternal immune homeostasis
- **sPIF** reduces spontaneous miscarriage to ~5%
- **sPIF** reduces inflammation LPS-induced fetal death ~2-fold
- sPIF promotes fetal weight – does not affect placental weight
- **sPIF** reduces placental inflammation and apoptosis (NALP3, Caspase-1)
- sPIF effect is integrated: reduces systemic inflammatory cytokines and locally protect the placenta

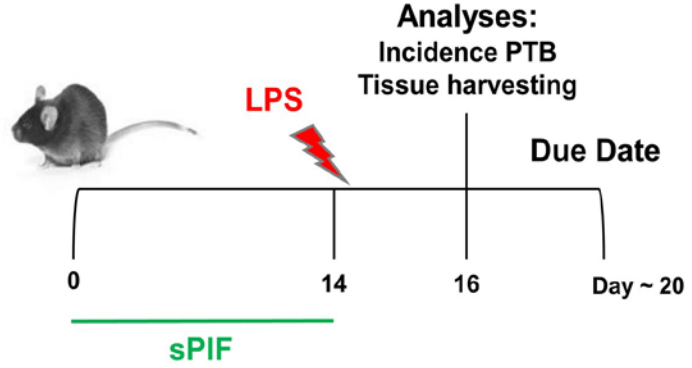
SRI-Pfizer President's Presenter's Award 2017

Mueller, M., Spinelli, M., Ornaghi, S., Schoeberlein, A., Bordey, A., Surbek, D., **Barnea, E.**, Paidas, M. (2018) PreImplantation Factor Promotes Neuroprotection by Modulating Long Non-Coding RNA H19 of the Neuronal Stem Cells. *Society for Reproductive Investigation 65th Annual Scientific Meeting* San Diego, CA

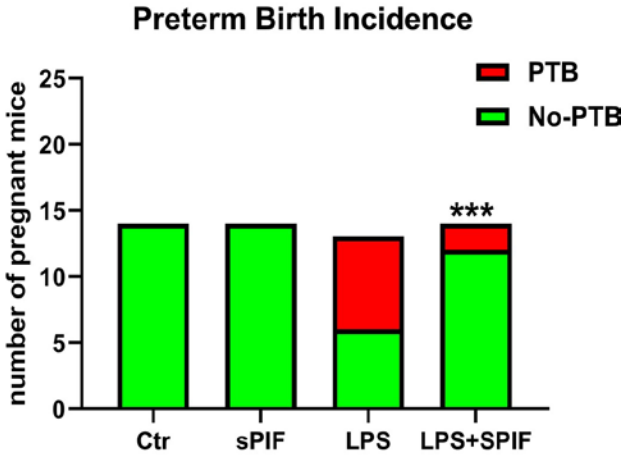
sPIF REDUCES INFLAMMATION INDUCED PREMATURE BIRTH

Protect fetal brain against inflammation (murine)

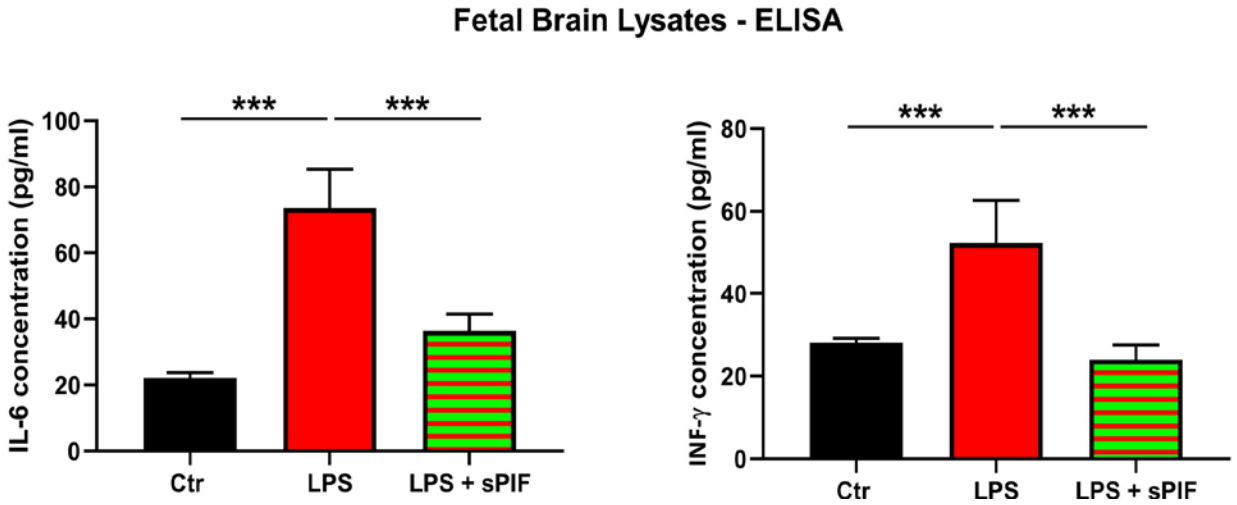
A.



B.



C.



Abstract

Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality and spontaneous PTB is a major contributor. The preceding inflammation/infection contributes not only to spontaneous PTB but is associated with neonatal morbidities including impaired brain development. Therefore, control of exaggerated immune response during pregnancy is an attractive strategy. A potential candidate is synthetic PreImplantation Factor (sPIF) as sPIF prevents inflammatory induced fetal loss and has neuroprotective properties. Here, we tested maternal sPIF prophylaxis in pregnant mice subjected to a lipopolysaccharides (LPS) insult, which results in PTB. Additionally, we evaluated sPIF effects in placental and microglial cell lines. Maternal sPIF application reduced the LPS induced PTB rate significantly. Consequently, sPIF reduced microglial activation (Iba-1 positive cells) and preserved neuronal migration (Cux-2 positive cells) in fetal brains. In fetal brain lysates sPIF decreased IL-6 and INFγ concentrations. In-vitro, sPIF reduced Iba1 and TNFα expression in microglial cells and reduced the expression of pro-apoptotic (*Bad* and *Bax*) and inflammatory (*IL-6* and *NLRP4*) genes in placental cell lines. Together, maternal sPIF prophylaxis prevents PTB in part by controlling exaggerated immune response. Given the sPIF'FDA Fast Track approval in non-pregnant subjects, we envision sPIF therapy in pregnancy.

Spinelli M, Boucard C, Di Nicuolo F, Haesler V, Castellani R, Pontecorvi A, et al. (2020) Synthetic PreImplantation Factor (sPIF) reduces inflammation and prevents preterm birth. PLoS ONE 15(6): e0232493. https://doi.org/10.1371/journal.pone.0232493

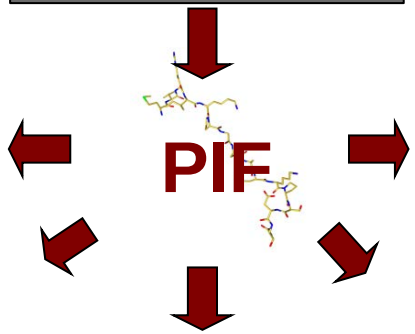
sPIF: TRANSLATIONAL IMPACT: PARADIGM SHIFT

REGULATES INFLAMMATION, IMMUNITY & TRANSPLANT

PIF TARGETS
Normal & Activated Immunity

PIF REGULATES
Oxidative Stress & Protein Misfolding

PIF ACTS
Locally & Systemically



Protects Against ARS
while
Regulating Inflammation

Reverses Paralysis
while
Promoting Neurogenesis

Reduces GVHD
while
Maintaining Beneficial GVL

Prevents Diabetes
while
Preserving Pancreatic Function

Reverses Brain Injury
while
Bolstering Neuroprotection

TM

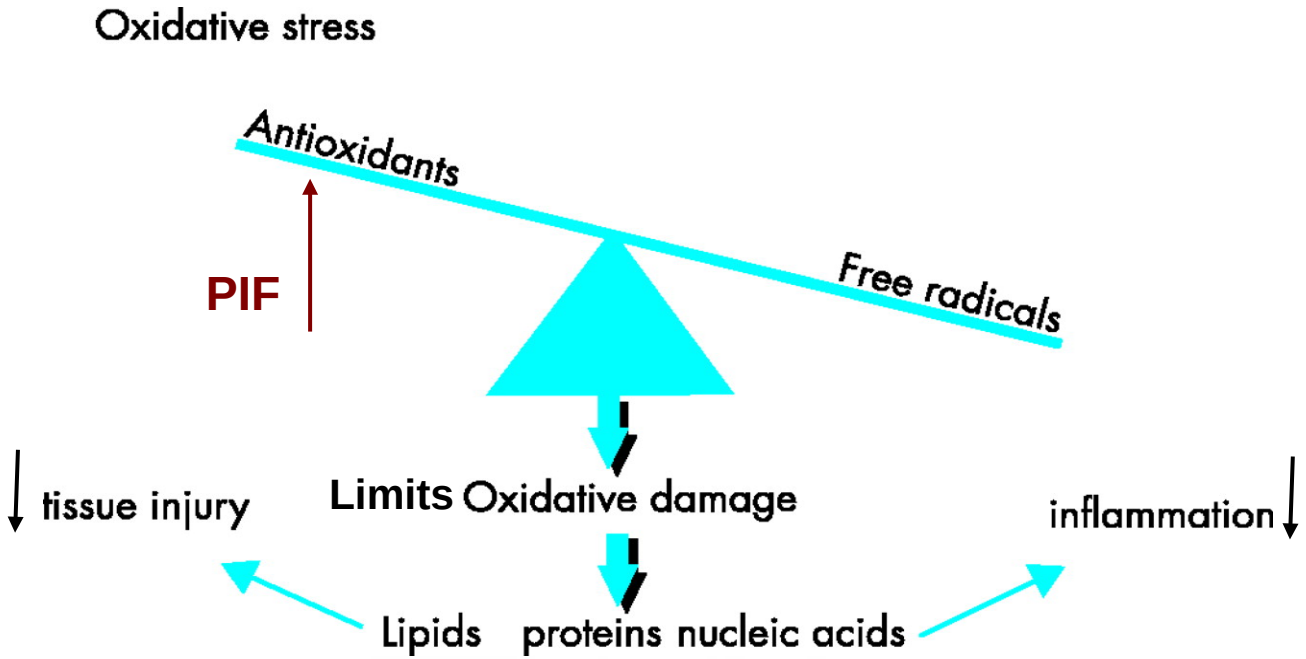
PIF MoA: LESSONS LEARNT FROM PREGNANCY

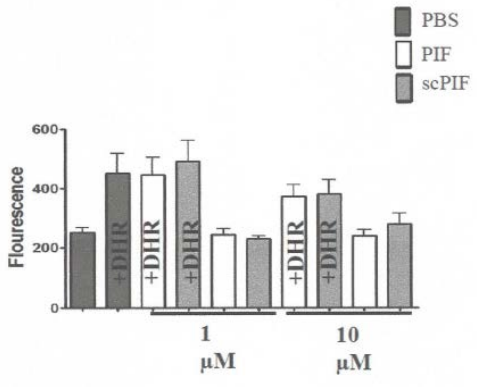
Achieve Immune Modulation & Transplant Acceptance

- ❑ Targets immune system *in vivo*
- ❑ Binds to specific receptors “ benign steroid”
- ❑ Regulates innate & adaptive immunity
- ❑ Exerts integrated local & systemic protection
- ❑ Protects embryo & reduces fetal demise
- ❑ Restores function
- ❑ Regenerative properties

sPIF REDUCES OXIDATIVE STRESS

Maintains Antipathogenic Protection





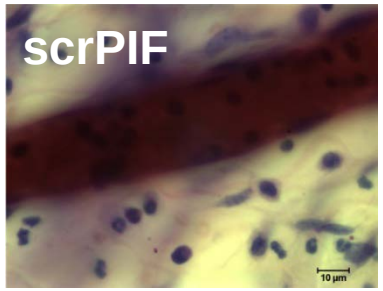
Almogi-Hazan et. al (2014). The Role of Oxidative Stress in Graft vs. Host Disease.In Oxidative Stress – Causes, Role in Diseases and Biological Effects. Nova Publishing, Inc. pp. 199-212

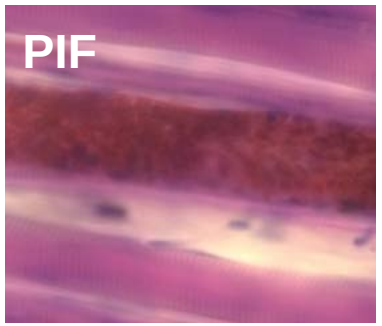
PreImplantation Factor Prevents Atherosclerosis via its Immunomodulatory Effects without Affecting Serum Lipids

Leucocytes preserve their ability to respond to challenge

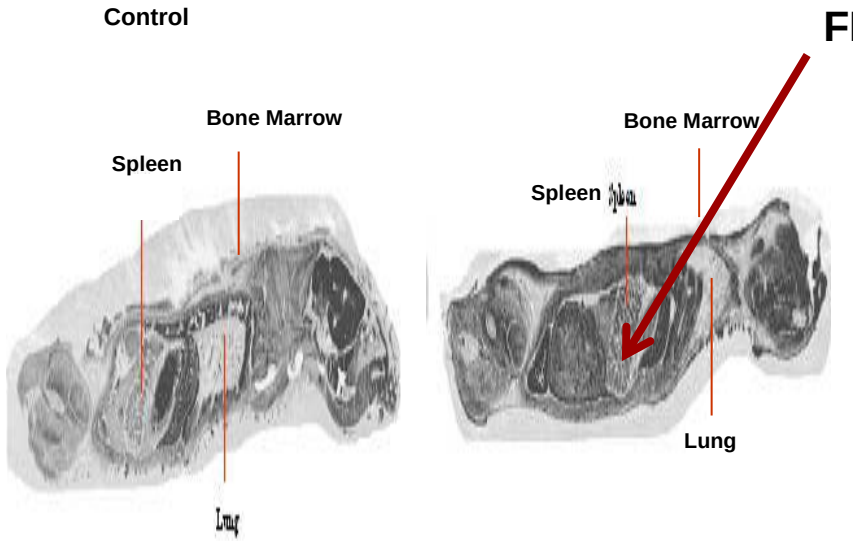
PIF TARGETS SYSTEMIC IMMUNITY *IN VIVO*

Reduced Neutrophil & Macrophage induced Inflammation

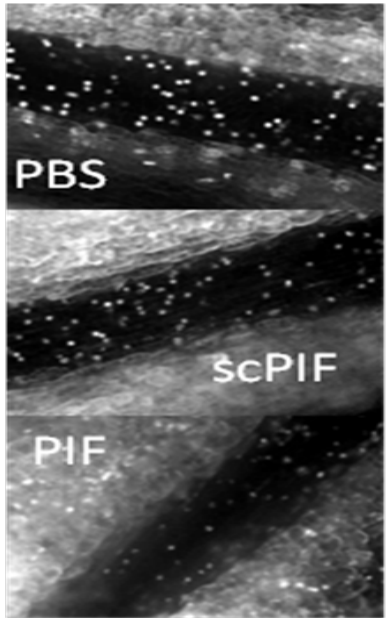




PIF blocks TNFa induced **neutrophils** vascular
Intravital microscopy

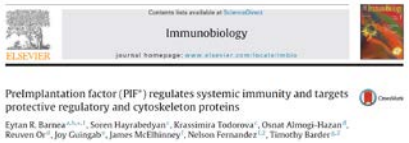


PIF uptake in spleen FITC –**PIF** (500 nM) injected IV with 5 min
Fluorescent analyzer (Typhoon`



PIF blocks LPS induced **macrophage** peritoneal inflammation
Intravital microscopy

- **sPIF** targets both local and systemic immunity for effective regulatory action (in vivo)
- **sPIF** binds to *systemic* immune cells, spleen, locally to bone marrow
- **PIF** has short half life, rapidly clears through kidney from circulation
- FITC-PIF binds innate all (CD14+ cells). In pregnancy the binding to lymphocytes(CD3+) increases
- Increased interaction of PIF with immune system may promote tolerance and anti-pathogen action



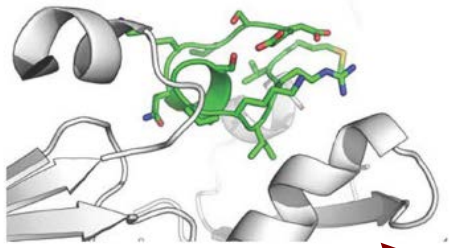
PreImplantation Factor Prevents Atherosclerosis via its Anti-inflammatory Effects without Affecting Serum Lipids

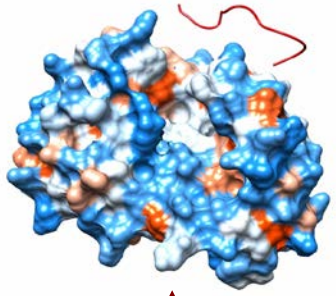
sPIF TARGETS AFFECTED ORGANS AND IMMUNE SYSTEM

Regulates Key Intracellular Proteins

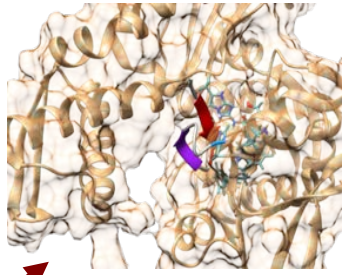
Kv1.3b K+ channel-cortisone binding site

PDI/Thioredoxin



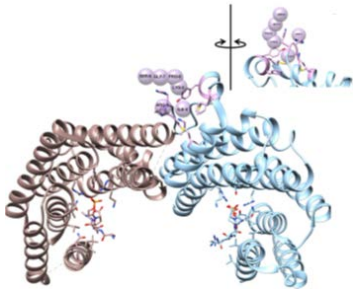


HSP - Heat Shock Proteins

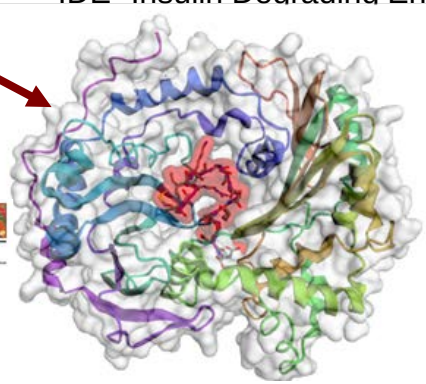


PIF

Scaffold Proteins 14-3-3



IDE- Insulin Degrading Enzyme



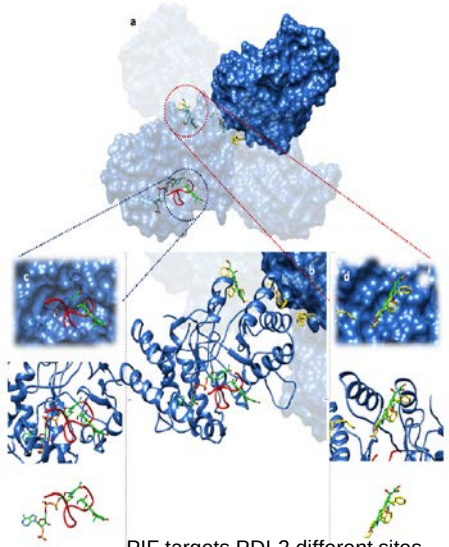
TM

sPIF ACTS AS A BENIGN STEROID

NON-STEROIDAL REGULATOR OF INFLAMMATION & IMMUNITY

Regulates Immune Response-Reduces K+ Flux

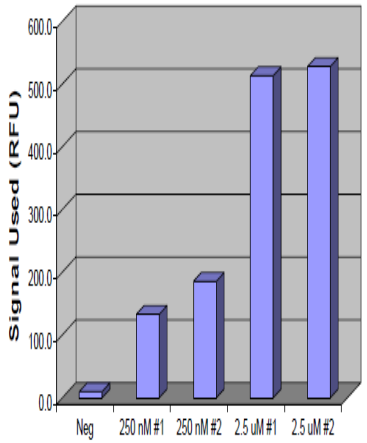
Receptor



PIF targets PDI-2 different sites

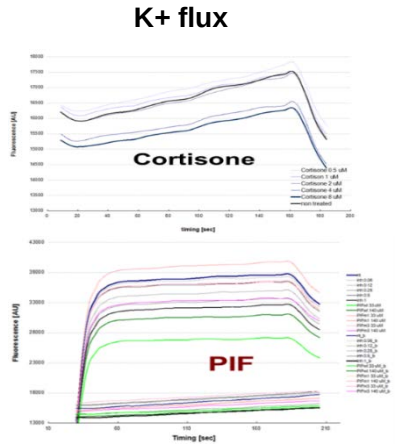
Cortisone CP330818

Binding Avidity



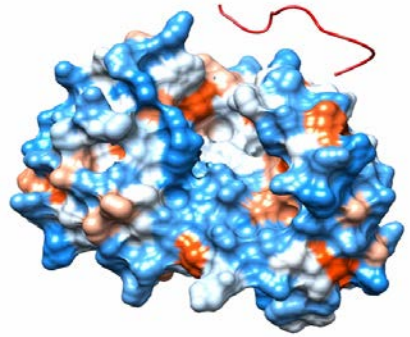
Dose dependent binding to PDI protein .
Proteoarray 9000 (Invitrogen)

Cell-Based Activity



PIF reduces K+ flux as cortisone
Dose dependent study
Jurkat cells

PIF TARGET

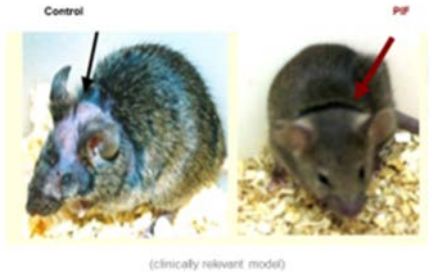


PreImplantation Factor Prevents Atherosclerosis via its Anti-inflammatory Effects without Affecting Serum Lipids

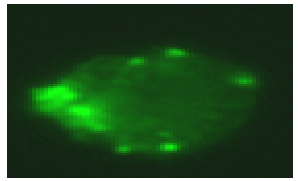
Yung Chih Chen[1], Jennifer Rivera[1], Melissa Fitzgerald[1,2], Christian Hausling[3], Ya-Lan Ying[3] Xiaowei Wang[1,2], Krassimira Todorova[3], Soren Hayrabedyan[4], Eytan R. Barnea[1], Kathelnz Peter[1,3]

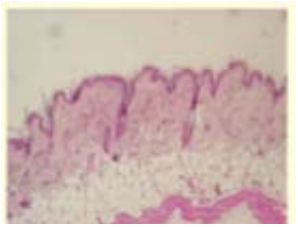
sPIF: GVHD; INTEGRATED LOCAL & SYSTEMIC EFFECTS

By Targeting the Immune System, PIF Achieves Protection of Susceptible Organs

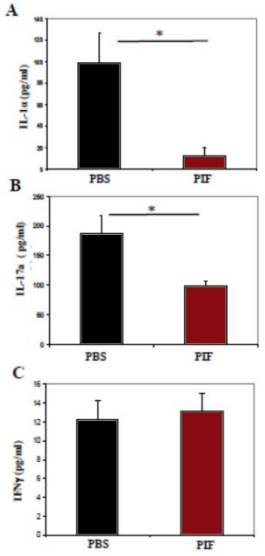


Inject **sPIF**





Skin ↓ ulcer



↓Circulating Th1/Th17

Liver ↓ inflammation

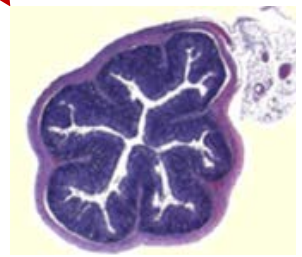
↓

Inos- oxidative stress

↓ ↓

IFN-γ/TNF-α/IL6

↓Ccr4/Ccl17



Colon ↓ ulcer

sPIF monotherapy

- Semi and allogeneic BMT
- 14 days
- Follow-up for 4 month

Results obtained using sPIF

- Prevent GVHD by protecting vulnerable areas: skin, liver and colon long-term
- Restore gut architecture
- Reduce fibrosis
- Promote skin healing
- Restore liver function
- Improve kidney function

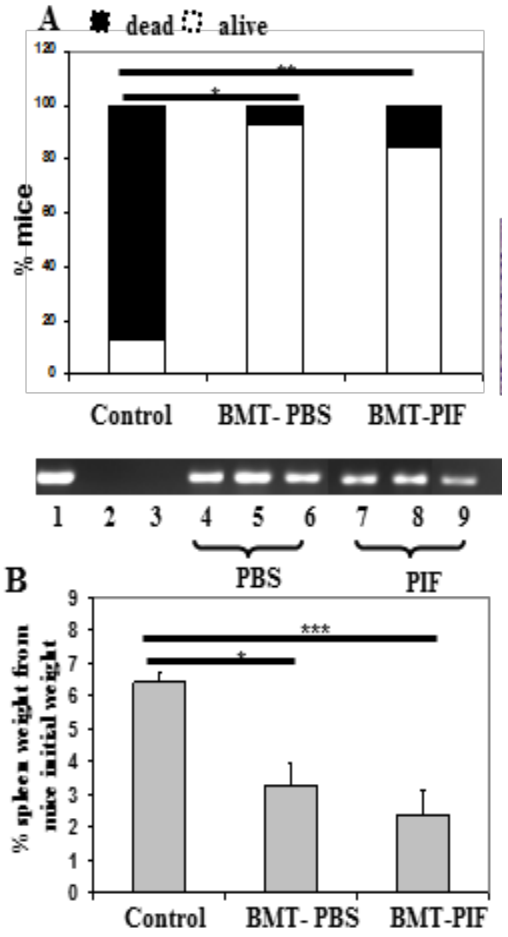
TM

Immune Regulation and Oxidative Stress Reduction by PIF Following Syngeneic or Allogeneic Bone Marrow Transplantation

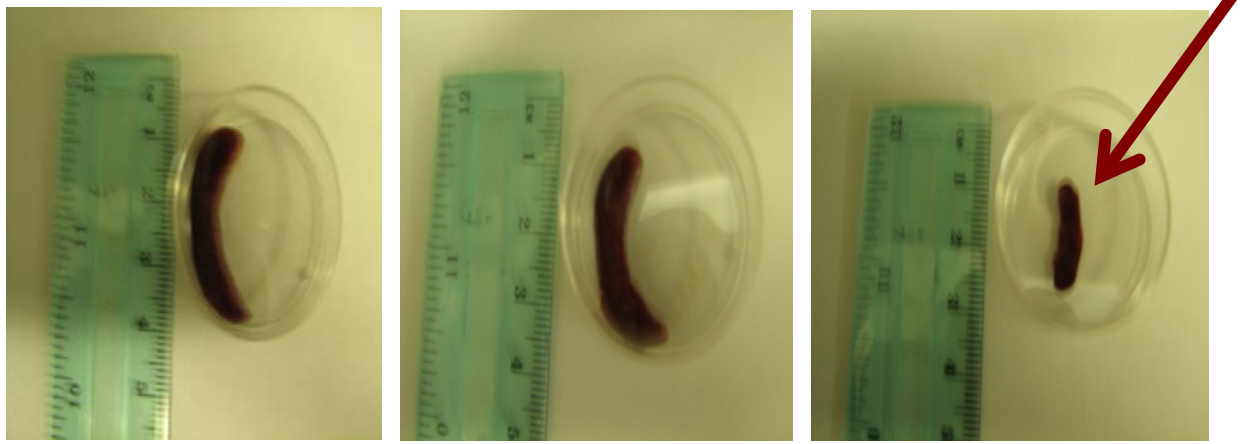
PIF Reduces Graft versus Host Disease (GVHD) by Regulating Immune Response and Lowering Oxidative Stress (murine)

PIF PRESERVES BENEFICIAL GVL & REDUCES DELETERIOUS GVHD

Reduces Mortality; Spleen Infiltration by BCL1 Leukemia



PIF Rx



Control | Autologous BMT | Mismatched BMT

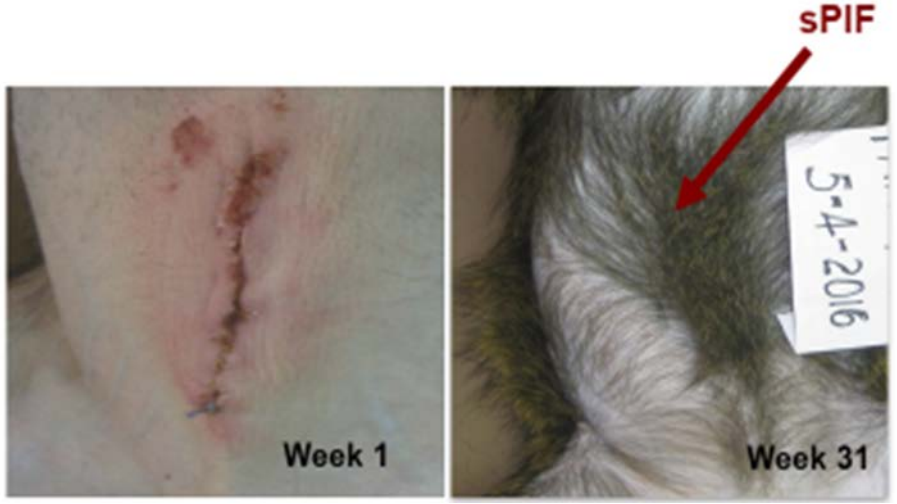
Biology

Preimplantation Factor Reduces Graft-versus-Host Disease by Regulating Immune Response and Lowering Oxidative Stress (Murine Model)

Yehudith Azar [1,†], Reut Shainer [1,†], Osnat Almogi-Hazan [1], Rachel Bringer [1], Susan R. Compton [2], Michael J. Paidas [3], Eytan R. Barnea [4,5,6,*,†], Reuven Or [1,†]

sPIF ACHIEVES ALLOTRANSPANT ACCEPTANCE- RETURN TO FUNCTION SCARLESS HEALING

Ovarian Tissue Allotransplant **NHP**



sPIF prevent scar formation promote hair growth

sPIF restores ovarian function- estrus

Ovarian allotransplant requires immune suppressive drugs
Surgery cause scar and internal adhesions / fibrosis
sPIF monotherapy NHP ovarian allotransplant 3w and f/y x2/w 5m.

sPIF restored ovarian function (estrus) long term

- **sPIF** led to scarless healing
- **sPIF** transplant
- Presence of ovarian follicle

TM

sPIF ADDRESSES ARS COMPREHENSIVELY

sPIF Rx pre/at/post Radiation Lethal & Sub-Lethal Damage. Full-Body & Local Exposure. Short & Long-Term.

sPIF PROTECTS AGAINST Radiation-Induced Disease ARS

Lethal H-ARS
- **Burn**
- **Neuro/Polytrauma**

Sublethal
- **Polytrauma**
- **GI-ARS/N-ARS**
- **Infection**

Delayed
- **BMT/CANCER**
- **Fibrosis**
- **Neurocognitive**

TM

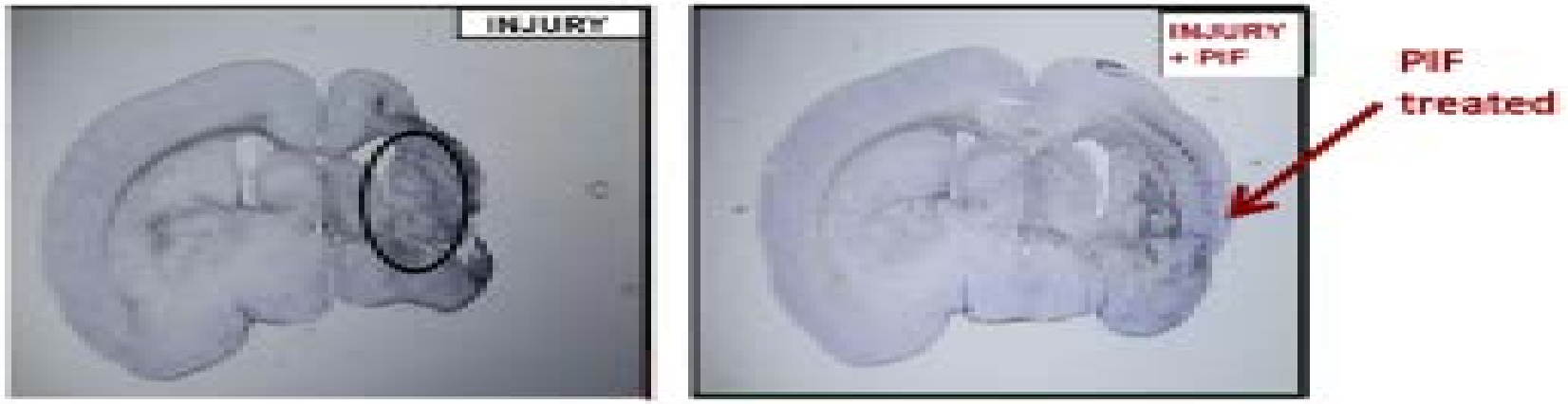
Oncotarget

PreImplantation Factor (PIF*) Therapy Provides Comprehensive Protection Against Radiation Induced Pathologies

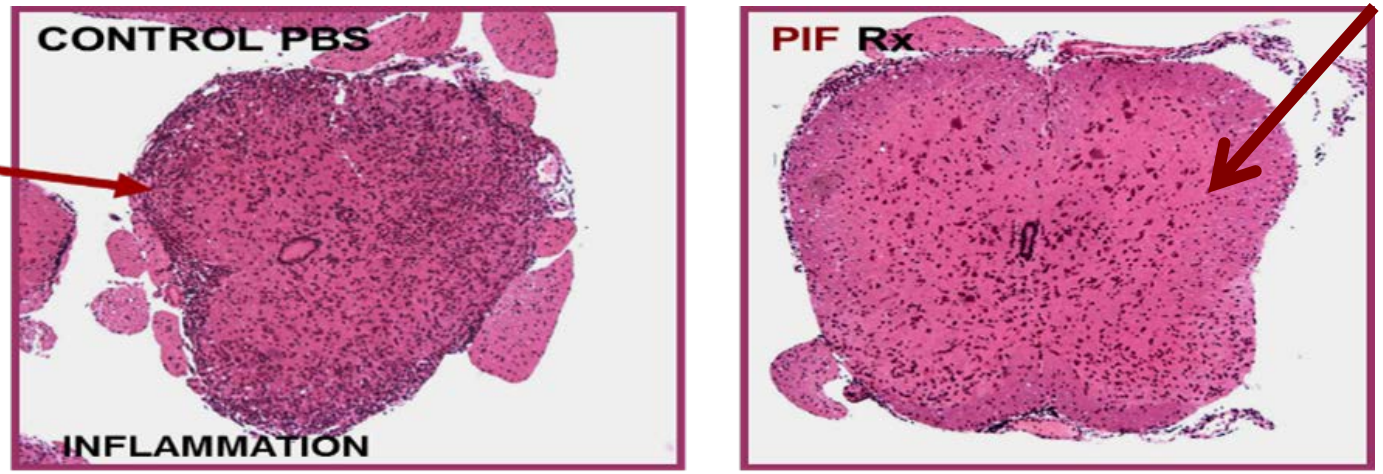
sPIF SHORT-TERM LOW-DOSE Rx BOLSTERS NEUROPROTECTION

Promotes Neural Repair (HIE Newborn) to Reduces Neuro Inflammation (Adult)

sPIF crosses intact BBB without degradation



sPIF Restore Brain Corpus Striatum



sPIF Reverses spinal cord inflammation

ARS alters neurological wellbeing

Newborn HIE complex neurotrauma model (murine)

- **sPIF** reverses brain damage
- **sPIF** promotes neural stem cells repair and proliferation

Adult neuroinflammation models: (murine)

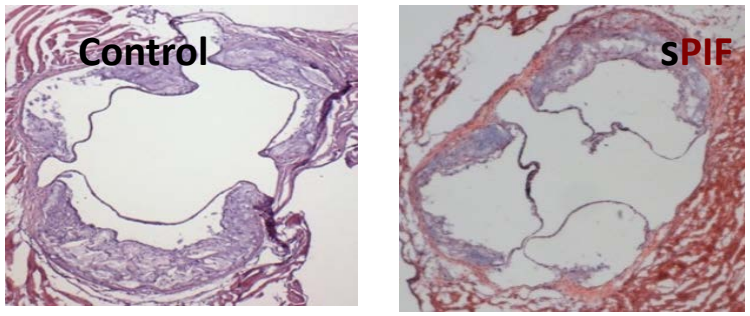
- sPIF reduces mortality
- sPIF reverses chronic paralysis
- sPIF promote brain remyelination
- sPIF reverse spinal cord inflammation
- sPIF reduces systemic inflammation
- sPIF reduces ALS symptoms

- **sPIF** directly targets the brain
- **sPIF crosses the Intact BBB**
- **sPIF remains intact in brain without degradation**

ICoM VIRTUAL
PNAS
PreImplantation Factor Promotes Neuroprotection by Targeting microRNA Let-7
Cell Death & Differentiation
PreImplantation Factor Bolsters Neuroprotection via Modulating Protein Kinase A and Protein Kinase C Signalling
SCIENTIFIC REPORTS
Synthetic PreImplantation Factor (sPIF) induces posttranslational protein modification and reverses paralysis in EAE mice
scientific reports
Murine glial progenitor cells transplantation and synthetic PreImplantation Factor (sPIF) reduces inflammation and early motor impairment in ALS mice
Oncotarget
PIF* Promotes Brain Re-myelination Locally while Regulating Systemic Inflammation- Clinically Relevant Multiple Sclerosis M Smegmatis Model
JCI insight
Preimplantation factor modulates oligodendrocytes by H19-induced demethylation of NCOR2

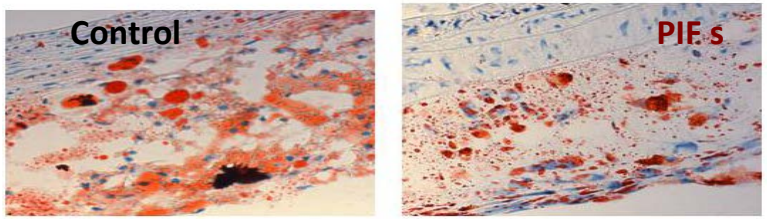
PIF PREVENTS ACCELERATED ATHROSCLEROSIS DEVELOPMENT

Reduced Plaque Formation Does Not Affect High Circulating Lipids

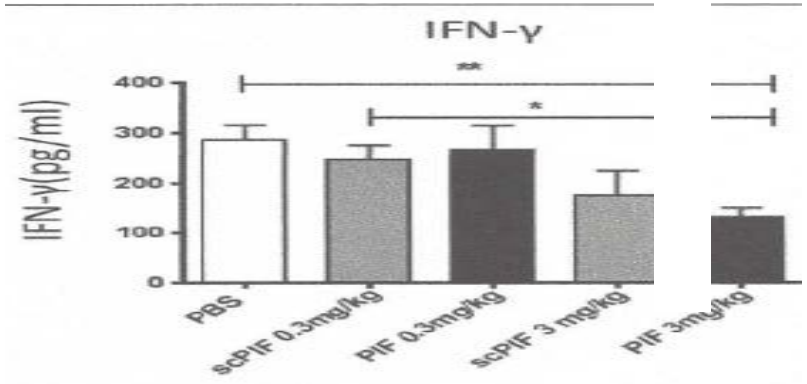


AORTIC ROOT (IHC)

SQ **PIF** prevents atherosclerosis.



Oil Red O staining (Fat deposits)



Animal Model: ApoE-deficient mice

High fat Western diet PIF Rx x2 w for 7 w

Summary

PreImplantation factor (PIF) is a 15-amino acid peptide endogenously secreted by viable embryos, regulating/enabling maternal (host) acceptance/tolerance to the "invading" embryo (allograft) all-while preserving maternal immunity to fight infections. Such attributes make PIF a potential therapeutic agent for chronic inflammatory diseases. We investigated whether PIF's immunomodulatory properties prevent progression of atherosclerosis in the hyper-cholesterolaemic ApoE-deficient murine model. Male, high-fat diet fed, ApoE-deficient ($ApoE^{-/-}$) mice were administered either PBS, scrambled PIF (0.3–3 mg/kg) or PIF (0.3–3 mg/kg) for seven weeks. After treatment, PIF (3 mg/kg)-treated $ApoE^{-/-}$ mice displayed significantly reduced atherosclerosis lesion burden in the aortic sinus and aortic arch, without any effect on lipid profile. PIF also caused a significant reduction in infiltration of macrophages, decreased expression of pro-inflammatory adhesion molecules, cytokines and chemokines in the plaque, and reduced circulating IFN-γ levels. PIF preferentially binds to monocytes/neutrophils. *In vitro*, PIF attenuated monocyte migration (MCP-1-induced chemotaxis assay) and *in vivo* in LPS peritonitis model. Also PIF prevented leukocyte extravasation (peritonitis thioglycollate-induced model), demonstrating that PIF exerts its effect in part by modulation of monocyte function. Inhibition of the potassium channel KCNAB3 (Kv1.3) and of the insulin degrading enzyme (IDE) was demonstrated as potential mechanism of PIF's immunomodulatory effects. In conclusion, PIF regulates/lowers inflammation and prevents atherosclerosis development without affecting circulating lipids. Overall our findings establish PIF as a strong immunomodulatory drug candidate for atherosclerosis therapy.

Keywords

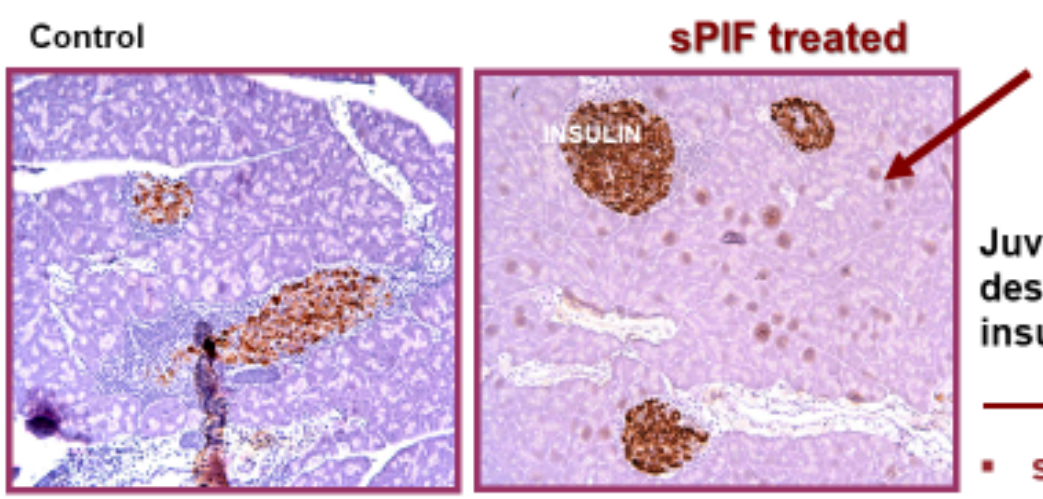
Atherosclerosis, immune cells, ApoE-deficient mice, PreImplantation Factor (PIF), macrophage, immunomodulatory therapy

PreImplantation Factor Prevents Atherosclerosis via its Anti-inflammatory Effects without Affecting Serum Lipids

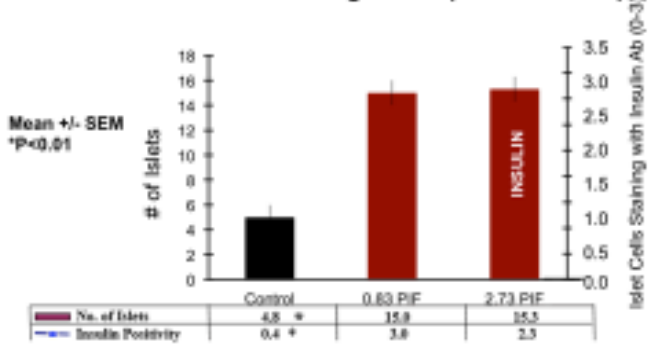
Yung Chih Chen[1], Jennifer Rivera[1], Melissa Fitzgerald[1,2], Christian Hausding[3], Ya-Lan Ying[1] Xiaowei Wang[1,2], Krassimira Todorova[4], Soren Hayrabedyan[5], Eytan R. Barnea[6], Karlheinz Peter[1,2,6]

sPIF PREVENTS JUVENILE DIABETES

Preserves Pancreatic Islets Insulin Expression



Short-term low-dose sPIF: long-term protection up to six months



Inoculation of diabetic immune cells in NOD mice adoptive transfer method

Juvenile diabetes causes islet destruction resulting in life-long insulin dependence

- **sPIF** prevents diabetes development
- **sPIF** normalizes glucose levels
- **sPIF** promotes pancreatic islets recovery

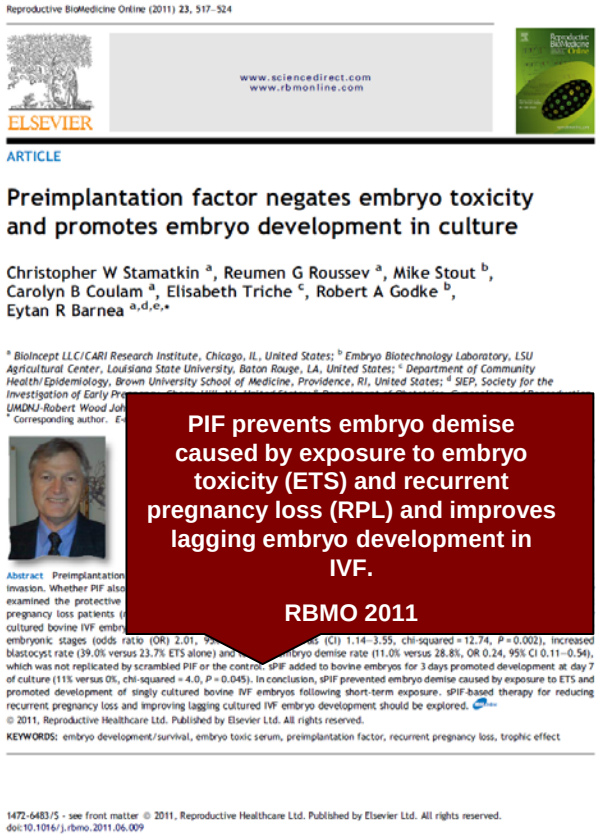
Endocrine

PIF Prevents Type I Diabetes Mellitus (T1DM) Development by Preserving Pancreatic Function (NOD)

TM

PIF: LOCAL & SYSTEMIC IMMUNE HOMEOSTASIS

Promote Embryo Development / Protect Against Pregnancy Loss



Reproductive BioMedicine Online (2011) 23, 517–524

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

ARTICLE

Preimplantation factor negates embryo toxicity and promotes embryo development in culture

Christopher W Stamatkin [a], Reumen G Roussev [a], Mike Stout [b], Carolyn B Coulam [a], Elisabeth Triche [c], Robert A Godke [b], Eytan R Barnea [a,d,e,*]

[a] BioIncept LLC/CARI Research Institute, Chicago, IL, United States; [b] Embryo Biotechnology Laboratory, LSU Agricultural Center, Louisiana State University, Baton Rouge, LA, United States; [c] Department of Community Health/Epidemiology, Brown University School of Medicine, Providence, RI, United States; [d] SIEP, Society for the Investigation of Early Pr… UMDNJ-Robert Wood Joh…
* Corresponding author. E-…

Abstract Preimplantation … invasion. Whether PIF also … examined the protective … pregnancy loss patients (… cultured bovine IVF embry… embryonic stages (odds ratio (OR) 2.01, 9… …s (CI) 1.14–3.55, chi-squared = 12.74, P = 0.002), increased blastocyst rate (39.0% versus 23.7% ETS alone) and … embryo demise rate (11.0% versus 28.8%, OR 0.24, 95% CI 0.11–0.54), which was not replicated by scrambled PIF or the control. sPIF added to bovine embryos for 3 days promoted development at day 7 of culture (11% versus 0%, chi-squared = 4.0, P = 0.045). In conclusion, sPIF prevented embryo demise caused by exposure to ETS and promoted development of singly cultured bovine IVF embryos following short-term exposure. sPIF-based therapy for reducing recurrent pregnancy loss and improving lagging cultured IVF embryo development should be explored.
© 2011, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: embryo development/survival, embryo toxic serum, preimplantation factor, recurrent pregnancy loss, trophic effect

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doi:10.1016/j.rbmo.2011.06.009

PIF prevents embryo demise caused by exposure to embryo toxicity (ETS) and recurrent pregnancy loss (RPL) and improves lagging embryo development in IVF.

RBMO 2011

Reproductive BioMedicine Online (2013) 26, 79–87

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

ARTICLE

Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy

Roumen G Roussev [a], Boris V Dons'koi [b], Christopher Stamatkin [a], Sivakumar Ramu [a], Viktor P Chernyshov [b], Carolyn B Coulam [a], Eytan R Barnea [c,d,e,*]

[a] CARI Reproductive Institute/BioInce… Obstetrics and Gynecology, Academy … Pregnancy, Cherry Hill, NJ, United St… of Obstetrics, Gynecology and Reprod…
* Corresponding author. E-mail address: …

Dr Eytan R B… endocrinology… clinical applic… Early Pregnan… and gynaecolo… Medical Schoo… Ministry/Jewi…

Abstract Embryo-secreted preimplanta… ment in humans by promoting implantat… tive in autoimmune disease models. s… suppression. This study examined the effect of sPIF on na… …cytotoxicity in 107 consecutive nonselected, nonpreg… nant patients with recurrent pregnancy loss (RPL) and 26 int… …tients (controls). The effects of sPIF, intravenous gamma immunoglobulin (Ig), Intralipid and scrambled PIF (PIFscr; negative control) on NK cell cytotoxicity to peripheral-blood cells were compared by flow cytometry of labelled-K562 cell cytolysis. The effects of sPIF and PIFscr on whole-blood NKCD69+ expression were also compared. In patients with RPL, sPIF inhibited NK cell cytotoxicity at doses of 2.5 and 25 ng/ml (37% and 42%) compared with PIFscr (18%; P < 0.001), regardless of the proportion of peripheral-blood NKCD56+ cells to lymphocytes. Pre-incubation of blood from infertile patients with sPIF for 24 h decreased NKCD69+ expression versus incubating with PIFscr (P < 0.05). In conclusion, sPIF inhibits NK cell cytotoxicity by reducing NKCD69 expression, suggesting a significant role in RPL patients.
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KEYWORDS: NK cell cytotoxicity, preimplantation factor (PIF), recurrent pregnancy loss (RPL), CD69, therapy

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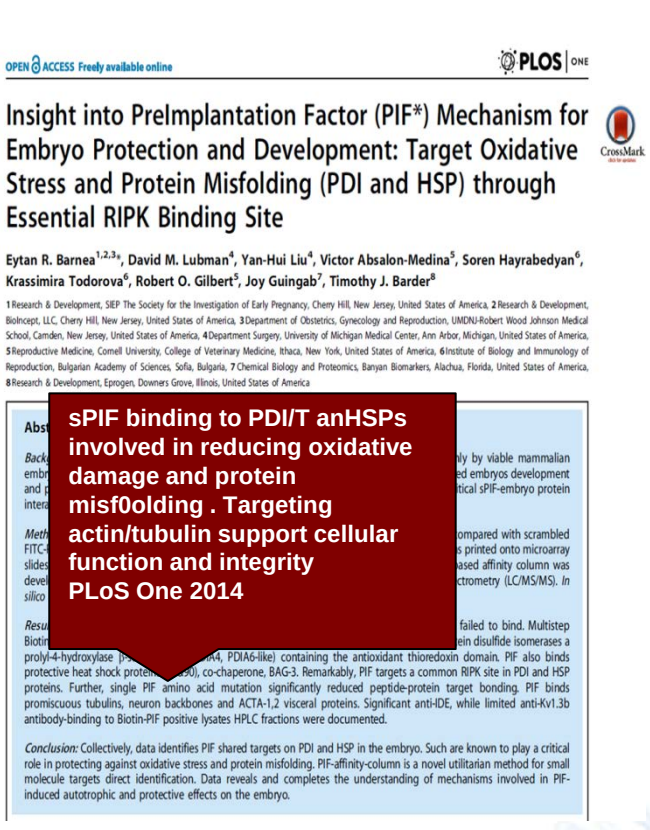
http://dx.doi.org/10.1016/j.rbmo.2012.09.017

sPIF inhibits NK cytotoxicity shown by reduction of NKCD69 expression, suggesting a significant role in RPL patients.

RBMO 2012



OPEN ACCESS Freely available online

PLOS ONE

Insight into PreImplantation Factor (PIF*) Mechanism for Embryo Protection and Development: Target Oxidative Stress and Protein Misfolding (PDI and HSP) through Essential RIPK Binding Site

Eytan R. Barnea[1,2,3*], David M. Lubman[4], Yan-Hui Liu[4], Victor Absalon-Medina[5], Soren Hayrabedyan[6], Krassimira Todorova[6], Robert O. Gilbert[5], Joy Guingab[7], Timothy J. Barder[8]

1 Research & Development, SIEP The Society for the Investigation of Early Pregnancy, Cherry Hill, New Jersey, United States of America, 2 Research & Development, BioIncept, LLC, Cherry Hill, New Jersey, United States of America, 3 Department of Obstetrics, Gynecology and Reproduction, UMDNJ-Robert Wood Johnson Medical School, Camden, New Jersey, United States of America, 4 Department Surgery, University of Michigan Medical Center, Ann Arbor, Michigan, United States of America, 5 Reproductive Medicine, Cornell University, College of Veterinary Medicine, Ithaca, New York, United States of America, 6 Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria, 7 Chemical Biology and Proteomics, Banyan Biomarkers, Alachua, Florida, United States of America, 8 Research & Development, Eprogen, Downers Grove, Illinois, United States of America

Abst…

Back… …ly by viable mammalian embr… …ed embryos development and p… …tical sPIF-embryo protein intera…

Meth… …ompared with scrambled FITC-… …s printed onto microarray slides… …ased affinity column was devel… …ctrometry (LC/MS/MS). *In silico*…

Resu… …failed to bind. Multistep Biotin… …ein disulfide isomerases a prolyl-4-hydroxylase … …4, PDIA6-like) containing the antioxidant thioredoxin domain. PIF also binds protective heat shock proteins (…90), co-chaperone, BAG-3. Remarkably, PIF targets a common RIPK site in PDI and HSP proteins. Further, single PIF amino acid mutation significantly reduced peptide-protein target bonding. PIF binds promiscuous tubulins, neuron backbones and ACTA-1,2 visceral proteins. Significant anti-IDE, while limited anti-Kv1.3b antibody-binding to Biotin-PIF positive lysates HPLC fractions were documented.

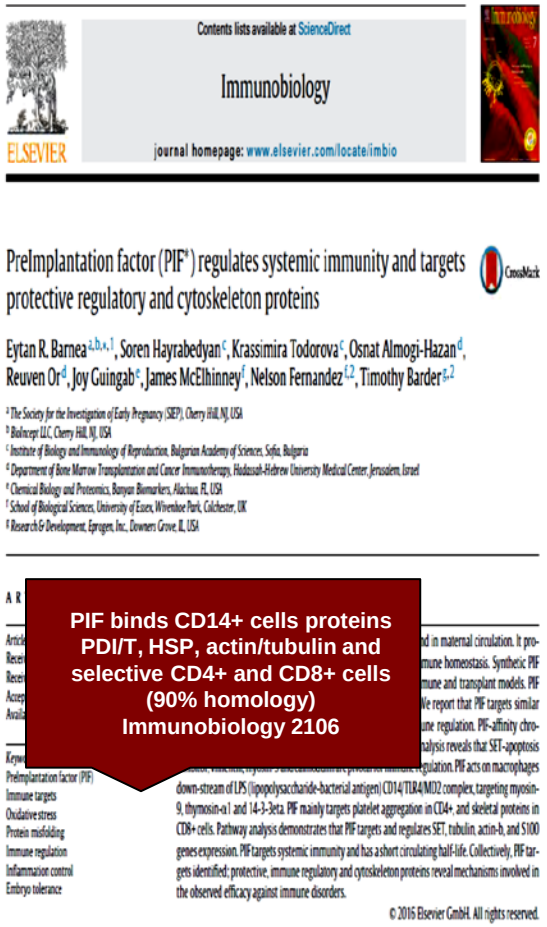
Conclusion: Collectively, data identifies PIF shared targets on PDI and HSP in the embryo. Such are known to play a critical role in protecting against oxidative stress and protein misfolding. PIF-affinity-column is a novel utilitarian method for small molecule targets direct identification. Data reveals and completes the understanding of mechanisms involved in PIF-induced autotrophic and protective effects on the embryo.

sPIF binding to PDI/T anHSPs involved in reducing oxidative damage and protein misf0olding . Targeting actin/tubulin support cellular function and integrity
PLoS One 2014

TM

PIF: TARGETED SYSTEMIC IMMUNE EFFECTS

Directly Regulate Immunity, Orchestrate Anti-Inflammation Response



Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

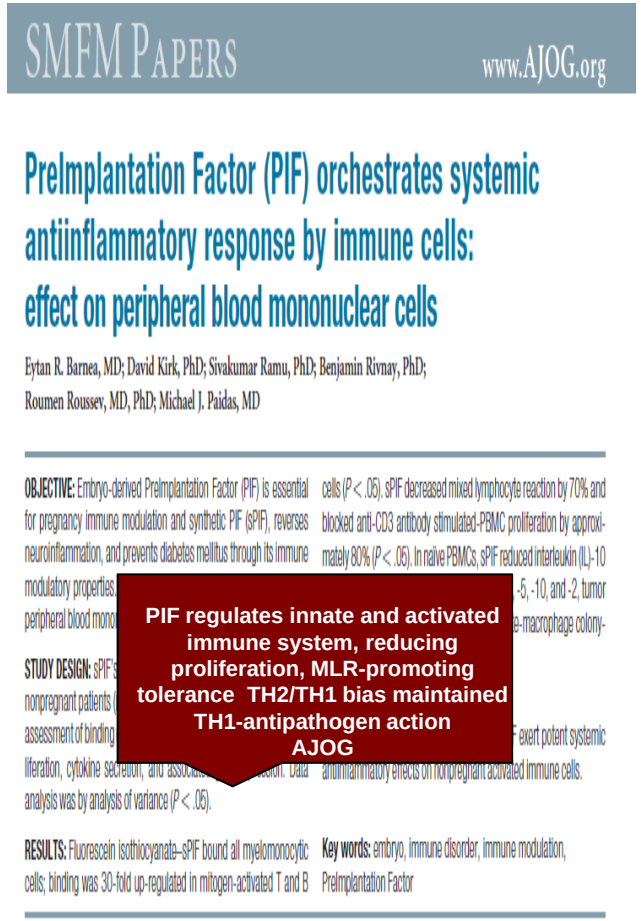
ELSEVIER

Preimplantation factor (PIF*) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins

Eytan R. Barnea[a,b,*,1], Soren Hayrabedyan[c], Krassimira Todorova[c], Osnat Almogi-Hazan[d], Reuven Or[d], Joy Guingab[e], James McElhinney[f], Nelson Fernandez[f,2], Timothy Barder[g,2]

[a] The Society for the Investigation of Early Pregnancy (SIEP), Cherry Hill, NJ, USA
[b] Bioincept LLC, Cherry Hill, NJ, USA
[c] Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria
[d] Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
[e] Chemical Biology and Proteomics, Banyan Biomarkers, Alachua, FL, USA
[f] School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, UK
[g] Research & Development, Epregen, Inc., Downers Grove, IL, USA

PIF binds CD14+ cells proteins PDI/T, HSP, actin/tubulin and selective CD4+ and CD8+ cells (90% homology) Immunobiology 2106



SMFM Papers www.AJOG.org

PreImplantation Factor (PIF) orchestrates systemic antiinflammatory response by immune cells: effect on peripheral blood mononuclear cells

Eytan R. Barnea, MD; David Kirk, PhD; Sivakumar Ramu, PhD; Benjamin Rivnay, PhD; Roumen Roussev, MD, PhD; Michael J. Paidas, MD

PIF regulates innate and activated immune system, reducing proliferation, MLR-promoting tolerance TH2/TH1 bias maintained TH1-antipathogen action AJOG

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

ELSEVIER

PIF direct immune regulation: Blocks mitogen-activated PBMCs proliferation, promotes T_H2/T_H1 bias, independent of Ca^{2+}☆

Eytan R. Barnea[a,b,*], David Kirk[c], Krassimira Todorova[d], James McElhinney[e], Soren Hayrabedyan[d], Nelson Fernández[e]

[a] The Society for the Investigation of Early Pregnancy (SIEP), Cherry Hill, NJ, USA
[b] Bioincept LLC, Cherry Hill, NJ, USA
[c] PharmMedInfo, Poulsbo, WA, USA
[d] Bulgarian Academy of Science, Sofia, Bulgaria
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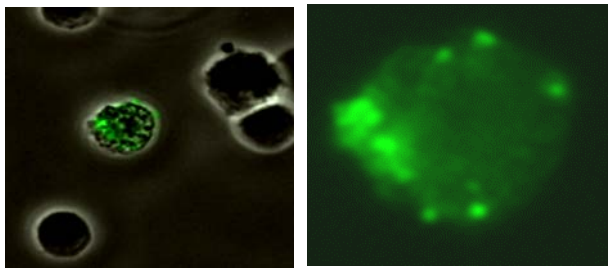
Direct effect
Systemic immune regulation
Embryo tolerance

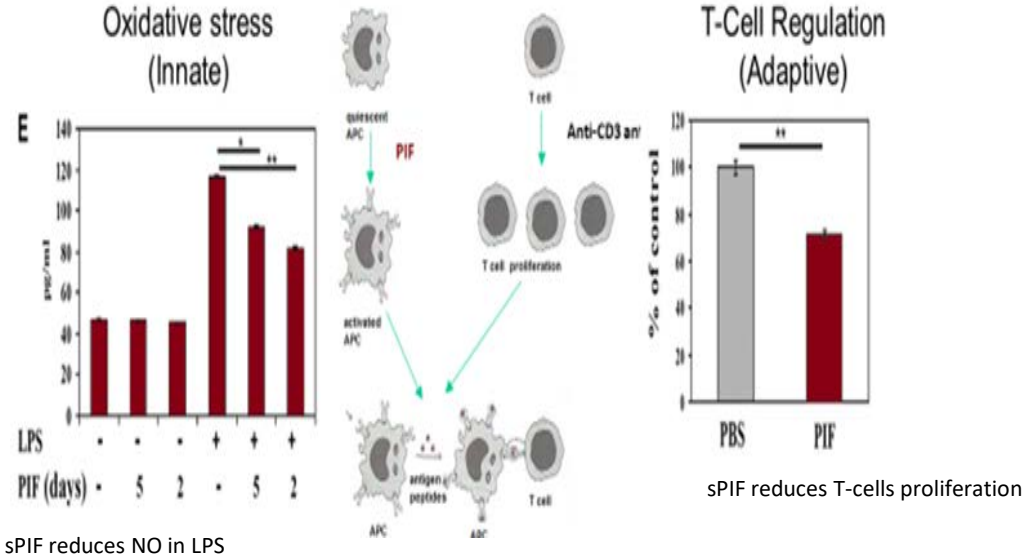
ABSTRACT

sPIF target immune cells and respond to specific stimulants Macrophages act as APC blocks T cells proliferation: increased CD2 Inhibition of CD58 expression Immunobiology 2015

TM

sPIF PROMOTES IMMUNE HOMEOSTASIS
Regulates Basal and Activated Immunity





sPIF reduces NO in LPS activated macrophages

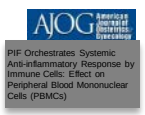
Monocytes differentiated with 200nM sPIF reduced Anti-CD3 activated T cells proliferation (co-culture)

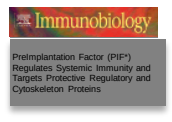
sPIF reduces T-cells proliferation

To prevent / avoid embryo / graft rejection, maternal immunity needs to be regulated by the viable embryo

- *Prior to pregnancy*, **sPIF** binds only basal systemic maternal immune cells
- *During pregnancy*, **sPIF** binding to adaptive immune cells reflects protective action

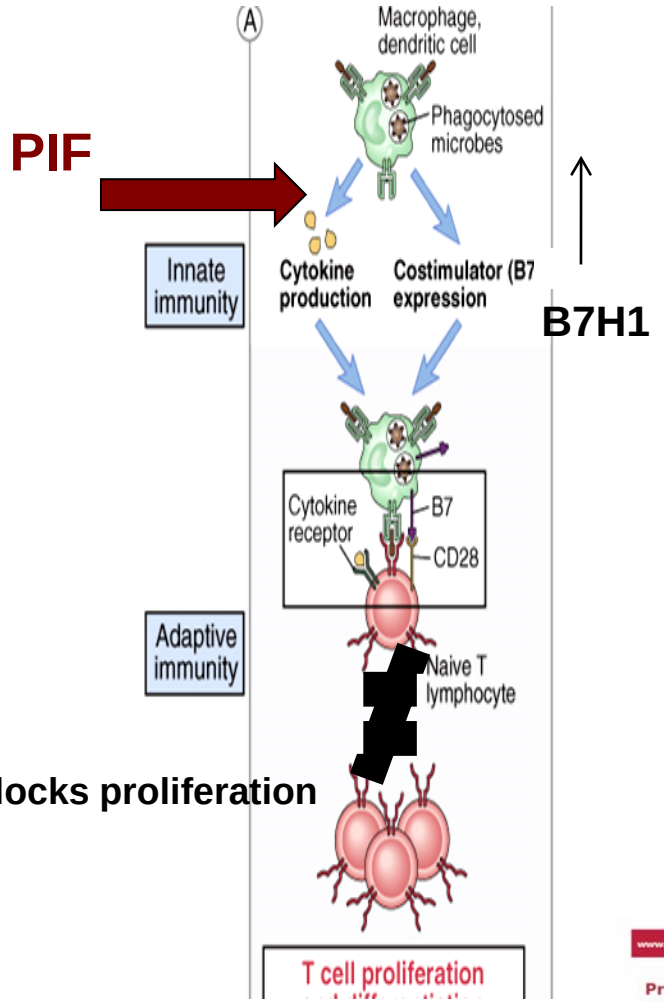
- **sPIF** targets basal (macrophage/neutrophils) and activated (lymphocytes)
- **sPIF** reduces inflammatory NO in LPS-activated macrophages
- sPIF activate macrophages acting as antigen presenting cells (APC) to block overactive lymphocytes
- Overall **PIF** achieves / preserves tolerance and maintains antipathogen activity

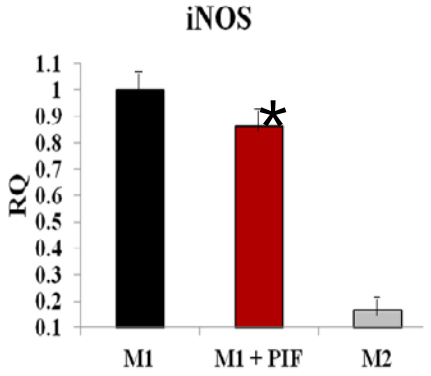


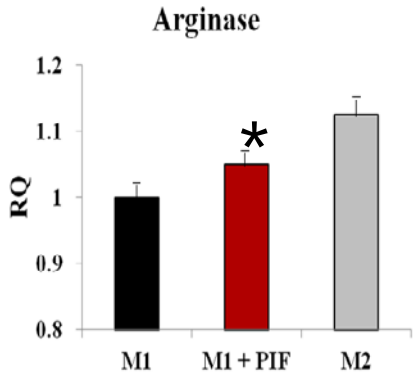


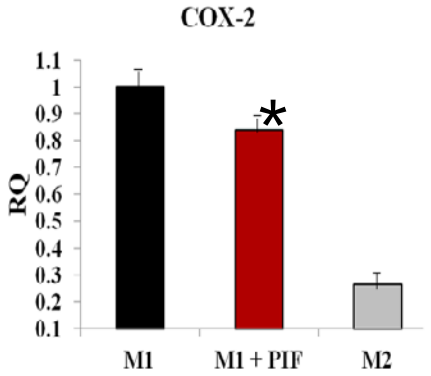
sPIF PROTECTS AGAINST OXIDATIVE STRESS

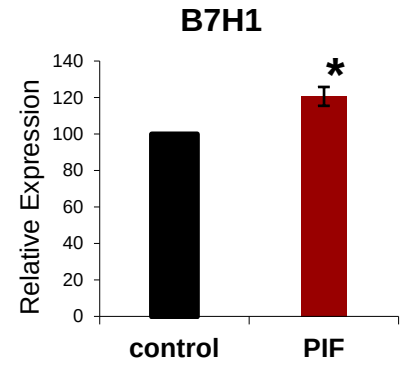
Shifts Macrophages to M2 Type







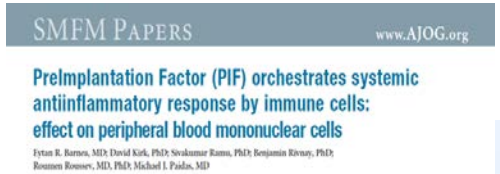





www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

PreImplantation factor (PIF) therapy provides comprehensive protection against radiation induced pathologies

Reut Shainer[1], Osnat Almogi-Hazan[1], Arye Berger[1], Liad Hinden[1], Martin Mueller[2,3], Chaya Brodie[4], Cedric Simillion[5], Michael Paidas[2], Eytan R. Barnea[6,7,*] Reuven Or[1,*]

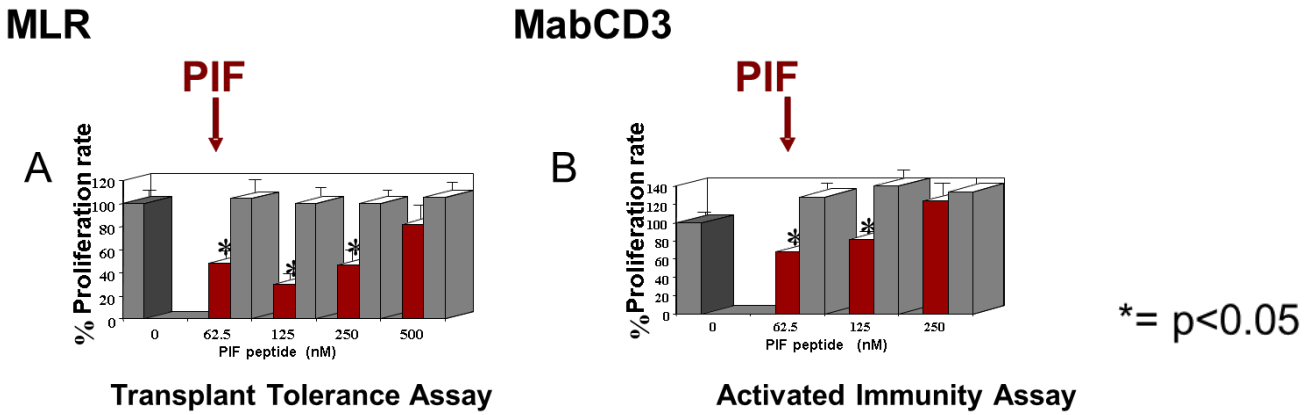

SMFM PAPERS www.AJOG.org

PreImplantation Factor (PIF) orchestrates systemic antiinflammatory response by immune cells: effect on peripheral blood mononuclear cells

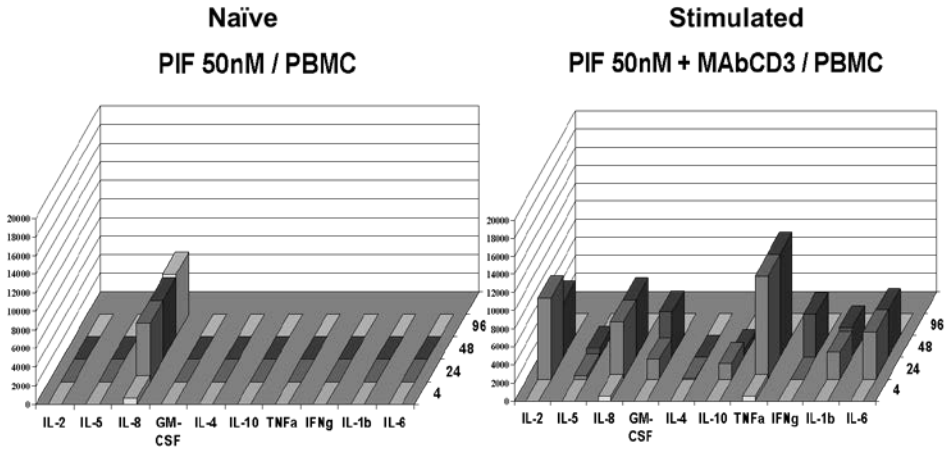
Eytan R. Barnea, MD; David Kirk, PhD; Sivakumar Ramu, PhD; Benjamin Rivnay, PhD; Roumen Rousev, MD, PhD; Michael I. Paidas, MD

sPIF REGULATES ACTIVATED IMMUNE RESPONSE

Reduces MLR & Proliferation Modulates Cytokine Secretion



PIF added to AIM V+/ -10 ug/ml DC3MAb (Mitomycin 100 ug/ml).
Empty bars: PIF / hatched bars: PIFscr (control, 3H Thymidine, Mean +/- SEM



PIF effect on CD3Mab-stimulated PBMCs
Cytokines measured (Luminex)

Confirmed by gene expression

SMFM Papers www.AJOG.org

Preimplantation Factor (PIF) orchestrates systemic antiinflammatory response by immune cells: effect on peripheral blood mononuclear cells

TAKE-HOME KEY MESSAGE

From Pregnancy to Immune Disorder Therapy
Integrated Local/Systemic Short term/Low dose/Long-term effect

- **sPIF** completed FDA Fast-Track Phase I safety clinical trial (NCT02239562)
- **sPIF** FDA chronic toxicology demonstrate safety
- **sPIF: Broad Immune Modulatory, Protective and Regenerative Properties**
  - **sPIF &** Promote Embryo Viability / Maternal acceptance/Prevent fetal demise/prematurity
  - **sPIF &** Target affected organs and systemic immunity
  - **sPIF &** Bind PDI and HSPs lower oxidative stress & protein misfolding
  - **sPIF &** Target Kv1b, -cortisone binding site reduce K+ -without suppression
  - **sPIF** & Local & Systemic Immune Homeostasis: Protection without Suppression
  - **sPIF &** Promote alo-BMT acceptance/ Prevent GvHD/ preserve beneficial GVL
  - **sPIF &** Promote alo-Ovarian transplant restore function/scarless healing (NHP)
  - **sPIF &** Protect against ARS acute, chronic
  - **sPIF &** Reverse Neurotrauma/Neuroinflammation
  - **sPIF &** Prevent Juvenile diabetes/ Reverse vascular inflammation

  - CONCLUSION: sPIF is safe suitable for chronic immune disorders therapy

TM

PIF PUBLICATIONS

Hormone and Metabolic Research

PreImplantation Factor (PIF*) Regulates Stress-Induced Adrenal Steroidogenesis and Anti-Inflammatory Cytokines: Potential Application for Bioartificial Adrenal Transplant

Pediatric Endocrinology Reviews

PIF and Endocrinology of Implantation and Establishment of Early Pregnancy: A Contemporary View

JARG asrm

Allogeneic Ovarian Transplantation using Immunomodulator PreImplantation Factor (PIF) as Monotherapy Restored Ovarian Function in Olive Baboon

Cellular Physiology and Biochemistry

Preimplantation Factor (PIF) Promotes HLA-G, -E, -F, -C Expression in JEG-3 Choriocarcinoma Cells and Endogenous Progesterone Activity

PLOS ONE

Synthetic PreImplantation Factor (PIF) Prevents Fetal Loss by Modulating LPS Induced Inflammatory Response

PLOS ONE

PreImplantation Factor in Endometriosis: A Potential Role in Inducing Immune Privilege for Ectopic Endometrium

Oncotarget Open Access Impact Journal

PreImplantation factor (PIF) protects cultured embryos against oxidative stress: relevance for recurrent pregnancy loss (RPL) therapy

Oncotarget Open Access Impact Journal

PIF Promotes Brain Re-myelination Locally while Regulating Systemic Inflammation – Clinically Relevant Multiple Sclerosis M Smegmatis Model

Cell Death & Disease nature

PreImplantation Factor is an Anti-Apoptotic Effector in Human Trophoblasts Involving p53 Signaling Pathway

Stem Cells and Development

Wharton's Jelly Mesenchymal Stem Cells Protect the Immature Brain in Rats by Modulating Cell Fate

Thrombosis and Haemostasis

PreImplantation Factor Prevents Atherosclerosis via its Immunomodulatory Effects without Affecting Serum Lipids

Immunobiology

PIF direct immune regulation: Blocks mitogen-activated PBMCs proliferation, promotes T_H2/T_H1 bias, independent of Ca^2

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

PreImplantation Factor (PIF*) Endogenously Prevents Preeclampsia: Promotes Trophoblast Invasion and Reduces Oxidative Stress

Oncotarget Open Access Impact Journal

PreImplantation Factor Therapy Provides Comprehensive Protection Against Radiation Induced Pathologies

birth defects research

Thrombosis During Pregnancy: Risks, Prevention, and treatment for Mother and Fetus. Harvesting the Power of Omic Technology, Biomarkers, and In Vitro or In Vivo Models to Facilitate the Treatment of Thrombosis

Immunobiology

PreImplantation Factor (PIF*) Regulates Systemic Immunity and Targets Protective Regulatory and Cytoskeleton Proteins

Pharmacology & Therapeutics

Immune Regulatory and Neuroprotective Properties of PreImplantation Factor (PIF*): From Newborn to Adult

Cell Death & Differentiation nature

PreImplantation Factor Bolsters Neuroprotection via Modulating Protein Kinase A and Protein Kinase C Signalling

PNAS of the United States of America

PreImplantation Factor Promotes Neuroprotection by Targeting microRNA Let-7

JOURNAL OF NEURODEVELOPMENTAL DISORDERS

PreImplantation Factor (PIF*) Promotes Embryotrophic and Neuroprotective Decidual Genes; Effect Negated by Epidermal Growth Factor

PIF PUBLICATIONS (CONT'D)

- PreImplantation Factor (PIF*) Promotes Human Trophoblast Invasion
- PreImplantation Factor (PIF*) Detection in Maternal Circulation in Early Pregnancy Correlates with Live Birth (bovine)
- Signalling Between Embryo and Mother in Early Pregnancy: Basis for Development of Tolerance
- The Role of Nitric Oxide Toxicity and Oxidative Stress in Graft vs. Host Disease
- Immune Regulation and Oxidative Stress Reduction by PIF Following Syngeneic or Allogeneic Bone Marrow Transplantation
- Reproduction and Autoimmune Disease (AD): Important Translational Implications from Embryo-Maternal Interaction
- PIF Inhibits Circulating NK Functional Activity & NK Activity by Reducing CD69 Expression: Implications for Recurrent Pregnancy Loss (RPL) Therapy
- PIF Reduces Graft versus Host Disease (GVHD) by Regulating Immune Response and Lowering Oxidative Stress (murine)
- Pregnancy and Multiple Sclerosis: A Beneficial Association. Possible Therapeutic Application of Embryo-Specific PIF
- PIF Orchestrates Systemic Anti-inflammatory Response by Immune Cells: Effect on Peripheral Blood Mononuclear Cells (PBMCs)
- PIF Promoting Role in Embryo Implantation: Increases Endometrial Integrin-α2β3, Amphiregulin & Epiregulin While Reducing Betacellulin Expression via MAPK in Decidua
- PIF Negates Embryo Toxicity and Promotes Embryo Development in Culture
- PIF Correlates with Early Mammalian Embryo Development (bovine & murine
- PIF Prevents Type I Diabetes Mellitus (TIDM) Development by Preserving Pancreatic Function (NOD)
- PIF Reverses Neuroinflammation While Promoting Neural Repair (EAE)
- Genomic and Proteomic Investigation of PIF's Impact on Human Decidual Cells
- PIF Promotes First Trimester Trophoblast Invasion
- Applying Embryo-Derived Immune Tolerance to the Treatment of Immune Disorders: Role of PIF

PIF PUBLICATIONS (CONT'D)

PLOS ONE | Insight into PreImplantation Factor (PIF*) Mechanism for Embryo Protection and Development: Target Oxidative Stress and Protein Misfolding (PDI and HSP) through Essential RIKP Binding Site

Oncotarget Open Access Impact Journal | The core sequence of PIF competes for insulin/amyloid β in insulin degrading enzyme – potential treatment for Alzheimer`s disease

Veterinary Medicine and Science | Preimplantation factor modulates acute inflammatory responses of equine endometrium

HEPATOLOGY COMMUNICATIONS | Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Trial of Synthetic PreImplantation Factor in Autoimmune Hepatitis

THE LANCET | FIGO Position paper: How to stop the Cesarean section Epidemic

AJRI | Insight into Early Pregnancy Events: The Emerging Role of the Embryo

Cell Death & Disease nature | sPIF Promotes Myoblast Differentiation and Utrophin Expression while Inhibiting Fibrosis in Duchenne Muscular Dystrophy via the H19/miR-675/let-7 and miR-21 Pathways

SCIENTIFIC REPORTS nature research | Synthetic PreImplantation Factor (sPIF) induces posttranslational protein modification and reverses paralysis in EAE mice

Citation: Spinelli M, Boucard C, Di Nicuolo F, Haesler V, Castellani R, Pontecorvi A, et al. (2020) Synthetic PreImplantation Factor (sPIF) reduces inflammation and prevents preterm birth. PLoS ONE 15(6): e0232493. https://doi.org/10.1371/journal.pone.0232493

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DISCLOSURE STATEMENT

Eytan R. Barnea, MD FACOG

Founder BioIncept™

I discovered PIF and dedicate my life to realize its intended potential development **from concept to clinics** with the aim and determination to bring PIF to patients. For this, I work for the last 15 years together with a trusted translational collaborating key opinion leader and commercial translation leader group.

The **PreImplantation Factor (PIF*)** is a 15aa ancestral endogenous peptide, evolutionary conserved, which mediates / regulates / adapts embryo's immunity and acceptance by its mother. It continues to protect the embryo-maternal interaction throughout viable pregnancy, in sickness and disease. (*PIF* Proprietary to BioIncept*)

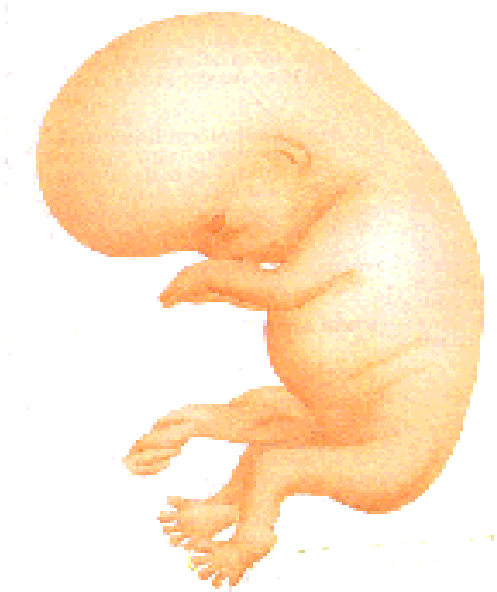
Synthetic PIF (sPIF* proprietary) allows adapting the use PIF's modulatory/regulatory features to treat pregnancy disorders and outside pregnancy to immune and transplant disorders. sPIF's action is addressing the immune imbalance and transplant acceptance rather than resorting to immune suppression or anti-rejection regimen.

I founded BioIncept, LLC with the mission to open a new, paradigm-shift diagnostic and treatment platform to regulate immunity, inflammation and transplant acceptance.

BioIncept is ready to enter Phase II clinical trials, was awarded Fast-Track status by the FDA and received Orphan Drug Designation(s) (ODD). Phase I first-in-human clinical trial was successfully completed showing that PIF has high safety margin, no toxicity and no deleterious drug-to-drug interaction.

The Barnea Family Limited Partnership is a substantial investor in BioIncept™ and Jacqueline H Barnea LLD, Co-founder of BioIncept® controls its shares.

THANK YOU



Statements in this presentation speak only as of the date they are made. Actual results from scientific tests could differ materially from those anticipated and there is no guarantee that clinical trials will yield results which are commensurate with preclinical testing done to date. Characterizations of the clinical benefits of products based on the PIF technology for the diagnosis or treatment may prove inaccurate, resulting in the estimated impact of PIF on the market for pregnancy and immune system diagnostics and therapeutics being overstated.

All information contained in this presentation is for the authorized recipient and intended for the limited purpose of an informational discussion about PreImplantation Factor (PIF) and synthetic PreImplantation Factor (sPIF^{R}).

This presentation does not constitute an offer to sell securities, which will only be made by BioIncept^{R} authorized persons to qualified persons by delivery, and acceptance, of a Confidential Private Placement Memorandum and specific documents referenced therein.