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Immune regulatory and neuroprotective properties of preimplantation factor: From newborn to adult

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ABSTRACT

Embryonic–maternal interaction from the earliest stages of gestation has a key, sustained role in neurologic development, persisting into adulthood. Early adverse events may be detrimental in adulthood. Protective factors present during gestation could significantly impact post-natal therapy. The role of PreImplantation Factor (PIF) within this context is herein examined. Secreted by viable early embryos, PIF establishes effective embryonic–maternal communication and exerts essential trophic and protective roles by reducing oxidative stress and protein misfolding and by blunting the nocive let-7 microRNA related pathway. PIF's effects on systemic immunity lead to comprehensive immune modulation, not immune suppression. We examine PIF's role in protecting embryos from adverse maternal environment, which can lead to neurological disorders that may only manifest post-nataly: Synthetic PIF successfully translates endogenous PIF features in both pregnant and non-pregnant clinically relevant models. Specifically PIF has neuroprotective effects in neonatal prematurity. In adult relapsing–remitting neuroinflammation, PIF reverses advanced paralysis while promoting neurogenesis. PIF reversed *Mycobacterium smegmatis* induced brain infection. In graft-vs.-host disease, PIF reduced skin ulceration, liver inflammation and colon ulceration while maintaining beneficial anti-cancer, graft-vs.-leukemia effect. Clinical-grade PIF has high-safety profile even at supraphysiological doses. The FDA awarded FAST-TRACK designation, and university-sponsored clinical trials for autoimmune disorder are ongoing. Altogether, PIF properties point to its determining regulatory role in immunity, inflammation and transplant acceptance. Specific plans for using PIF for the treatment of complex neurological disorders (ie. traumatic brain injury, progressive paralysis), including neuroprotection from newborn to adult, are presented.

Contents

Abbreviations: AFP, alpha fetoprotein; BBB, blood brain barrier; BMT, bone marrow transplant; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; EGF, epidermal growth factor; ET, embryo transfer; EVT, extravillous trophoblasts; FDA, Food and Drug Administration; FITC, Fluorescein isothiocyanate; FTDC, first trimester decidual cells; GA, gestational age; GMP, good manufacturing practices; GVHD, Graft vs. host disease; HESC, human endometrial stromal cells; HI, hypoxic–ischemic; HPLC, high performance liquid chromatography; IDE, insulin degrading enzyme; IHC, immunohistochemistry; IVF, in vitro fertilization; IVIG, intravenous gamma globulin; MET, mesenchymal epithelial transformation; MS, multiple sclerosis; NK, natural killer cells; NOD, non-obese diabetic; PBMCs, peripheral blood mononuclear cells; PDI, protein-disulfide-isomerase; PIF, PreImplantation Factor; PVL, periventricular leucomalacia; ROS, reactive oxygen species; RR, relapsing/remitting; SC, spinal cord.

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1. Introduction

1.1. PreImplantation Factor (PIF) is associated with viable embryos

When sperm and egg successfully interact, a fully formed mammal emerges with the capacity to live outside the uterus after delivery and throughout its life. From the two cells that create the zygote, 287 different cell types will ultimately form. Post-fertilization embryo development proceeds as ordered where two cells grow to four, eight, and sixteen until the embryoblast and trophoblast swiftly become distinct from each other. Embryonic programming is characterized by an order leading to emerging properties and yielding segmented organs (Barnea & Schofield, 1996). Once differentiated, cells specific to each organ will not usually be found elsewhere in the body, only in that specific organ. Thus, the embryo's emerging properties will effectively transpose structure to function.

At the start, the ratio of trophoblast to embryoblast cells is >500:1; by the end of human gestation this ratio inverts significantly, as the newborn size is approximately seven-fold greater than that of the supporting placenta (Barnea, 1997b; Barnea & Barnea, 1997). The embryoblast appears almost dormant until implantation and then its multiplication and differentiation occur very rapidly, leading to the development of the somatic body (Barnea, 1997a). On the other hand, the trophoblast, which led the implantation process, will progress to trophoblastic cells that are loosely collected cytotrophoblasts (ie. early syncytiotrophoblast) and extravillous trophoblast — the invading cells (Barnea, 2000; Barnea & Levine, 2000). During embryogenesis the cytotrophoblast will become the syncytiotrophoblast (a collection of multinucleated cells). Further, blood vessels derived from the embryo will make the villi vascular. The maternal vessels will then penetrate the intervillous spaces. Placental vessels thus enable effective trophoblast/uterus nutrient exchange.

Throughout development, it is the embryo, and later the fetal/placental unit, which will condition the maternal organism. Thus pregnancy is an embryo-centric rather than materno-centric endeavor (Rossant, 2001; Barnea, 2007c). For example, when an embryo is not viable, the maternal organism, in general, fails to undergo the significant adaptive changes expected with a viable embryo. Moreover, the embryo can implant outside the uterus (i.e. tubal ectopic pregnancy or abdominal pregnancy) and nonetheless cause maternal recognition/adaptation, a further illustration supporting the embryo-centric view. Thus, the uterus, although a preferred site of implantation, is not mandatory for embryonic viability.

Pregnancy is initiated by a maternal recognition which occurs prior to implantation, when the zygote is still surrounded by the semi-permeable zona pellucida. This is followed by a maternal adaptation to pregnancy in which an intimate embryo/trophoblast maternal interaction takes place and then continues until delivery. There are robust data strongly suggesting that the signal to initiate parturition is fetoplacental derived and not maternally driven (Barnea, 2007c).

The initiation of embryonic/maternal cross talk is evidenced in the maternal organism remarkably early. The first event is margination of platelets which occurs within 6 h of conception (O'Neill, 1985a). Thus, the immune environment is immediately altered as a result of the zygote's forming and emitting specific signals. Further evidence for this comes from changes in regulatory T-cells prior to implantation (Teles et al., 2013). These Tregs (CD25+ CD4+) express FoxP3 mRNA. Expression of these cells is enhanced in the presence of the viable embryo, and thereby facilitates maternal tolerance and adaptation to pregnancy.

During natural conception, the semen possesses immune regulating compounds. In contrast, following IVF and embryo transfer, these compounds are not operative, yet tolerance occurs nonetheless. In addition, the embryo in culture is self-sustaining since it can be cultured without any serum or growth factors. Thus self-development through autocrine factors occurs. Earlier studies have focused on early pregnancy factor (EPF), which is a chaperone, and also on platelet activating factor; however, neither are pregnancy-specific (O'Neill, 1985b; Athanasas-Platsis, Somodevilla-Torres, Morton, & Cavanagh, 2004; Ohnuma, Ito, Takahashi, Nambo, & Miyake, 2004; O'Neill, 2005). Additional compounds and several growth factors were also associated with maternal recognition of pregnancy, however again none are embryo-specific (Aplin & Kimber, 2004). HLA-G a pro-tolerance factor also does not appear to be essential for pregnancy (Criscuoli et al., 2005). Agents that protect the embryo against apoptosis (IGF-1, gonadotropin releasing hormone analog-1 and embryotrophic factor 3 derived from oviduct) have been described (Xu et al., 2004; Kawamura et al., 2005; Jousan, Oliveira, & Hansen, 2008). As for the endometrium, extensive investigations have been pursued to determine factors that can promote implantation. This was mainly, but not exclusively, focused on the sex steroids, estrogen and progesterone. The role of IGF-BP1, osteopontin, prostaglandin, and interleukin-1 has also been examined (Simon et al., 1994; Brar, Frank, Kessler, Cedars, & Handwerger, 1997; Gellersen & Brosens, 2003; Johnson, Burghardt, Bazer, & Spencer, 2003; Ghosh, Bell, & Sengupta, 2004; Kennedy, Gillio-Meina, & Phang, 2007; Das et al., 2009; Bombail et al., 2010; Mueller et al., 2015).

It became important to establish the existence and function of pregnancy-specific (particularly embryo-specific) factors that have the essential role as immune modulators and transplant acceptance compounds.

1.2. PreImplantation Factor identification and chemistry

It is our view that pregnancy, as a unique milieu should at least be initiated, if not primarily driven, by signaling derived from a viable embryo, (Barnea, 2014).

The realization that prior to implantation the maternal system is aware of the embryo presence without any direct contact, combined with evidence that the maternal immune system is already altered by that time, led us to the identification of factors responsible for early maternal recognition (Barnea & Coulam, 1997). Our premise was that factors involved should be embryo-specific and expressed only by viable embryos (Barnea & Sharma, 2007; Barnea, 2014). Whether the agents secreted by the embryo are identical to those observed in the maternal circulation was investigated. The specific embryo secretory product(s) that was identified, which distinguished between viable and non-viable embryo, became known as PreImplantation Factor (PIF).

Earlier studies have enabled the development of a novel rosette-based assay to identify the presence of PIF activity in murine and human embryo culture media as well as in the maternal circulation. We showed an increase in PIF activity as embryos were being cultured up to blastocyst the stage. We also found that PIF's increasing concentration in the maternal circulation was independent of progesterone or bHCG. This suggested that PIF detection in the maternal circulation could be a novel biomarker for diagnosing viable pregnancy (Barnea,