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Prolactin-like protein-A is a functional modulator of natural killer cells at the maternal–fetal interface

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Abstract

Natural killer (NK) cells are the predominant lymphocytes present in healthy rodent and human implantation sites. In the rat, the expansion, differentiation and subsequent migration of NK cells away from the developing chorioallantoic placenta coincide with the expression of a novel pregnancy- and trophoblast cell-specific cytokine, prolactin (PRL)-like protein A (PLP-A). PLP-A specifically binds to uterine NK cells but does not appear to utilize receptor systems for PRL. In the present report, we show that PLP-A interactions with NK cells are not mediated by receptors utilized by known modulators of NK cell function, including interleukin-2, interleukin-7, interleukin-12, and interleukin-15 (IL-15). Uterine NK cells respond to PLP-A or IL-15 with an increase in intracellular calcium mobilization. In contrast, PLP-A, unlike IL-15, effectively suppresses the ability of NK cells to produce interferon-γ (IFNγ), a key mediator of NK cell function. Placental PLP-A expression is reciprocal to mesometrial decidua expression of IFNγ. Increased expression of PLP-A by the placenta coincides with the decline of IFNγ content in the mesometrial decidua adjacent to the placenta. In summary, trophoblast cell-derived PLP-A contributes to the regulation of NK cells at the maternal–fetal interface to ensure appropriate embryonic growth and development.
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Keywords: Prolactin-like protein-A; Placenta; Trophoblast; Natural killer cell; Interleukin-15; Interferon-γ

1. Introduction

Normal progression of growth and maturation of the embryo/fetus requires concomitant adjustments in maternal physiology, including those related to the vasculature and immune system. Hemochorial placentation, as occurs in both primates and rodents, results in the establishment of a close connection between maternal and fetal tissues (Enders and Welsh, 1993). This close connection facilitates the exchange of nutrients and wastes at the expense of an increased risk of attack by the maternal immune system. Trophoblast cells of the placenta produce hormones and cytokines that are proposed to be involved in reprogramming maternal physiology for the benefit of the fetus. In rodents, the placenta produces a large collection of protein hormones that belong to the prolactin (PRL) family (Soares et al., 1998; Soares and Linzer, 2001). The PRL family consists of proteins encoded by at least 19 genes in the rat and 26 genes in the mouse (Soares and Linzer, 2001; Wiemers et al., 2003). One member of the PRL family, PRL-like protein-A (PLP-A) specifically interacts with uterine natural killer (NK) cells located within the uteroplacental compartment (Müller et al., 1999).

Uterine NK cells are the most prominent leucocytes present at the implantation sites of primates, rodents, ruminants, and pigs (Peel, 1989; Head, 1996; Croy et al., 1998; Slukvin et al., 2001; Moffett-King, 2002). Following implantation in the rat and mouse NK cells expand in number and differentiate in the mesometrial decidua adjacent to the chorioallantoic placenta (Head, 1996; Croy et al., 1998). Phenotypically, uterine NK cells undergo a gestational dependent transformation. After midgestation, NK cells are conspicuous in their relative

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absence of cytolytic activities and their enhanced production of specific bio-effector molecules (Croy et al., 1998). NK cells are also redirected away from the developing placenta and contribute to the formation of a new highly vascular structure, the metrial gland, which is embedded in the mesometrial myometrium (Peel, 1989). Uterine NK cells have been proposed to participate in immunological adjustments of pregnancy, the establishment of chorioallantoic placenta, and the modification of uterine vasculature (Croy et al., 1998; Croy et al., 2000; Liu and Young, 2001). The absence of NK cells, as in Tgε26 mice, is associated with reproductive deficits, which include edematous decidua, decidual vascular pathology, decreased placental size, increased fetal loss and low birth weight (Guimond et al., 1997, 1999). There is also some evidence suggesting aberrant NK cell activity as underlying the etiology of spontaneous abortions and preeclampsia (Stallmach et al., 1999).

The regulation of uterine NK cell trafficking and uterine NK cell activities at the maternal–fetal interface is not well understood. In general, NK cell activation can be influenced by cytokines and cell–cell interactions and is accompanied by a rapid intracellular mobilization of calcium and secretion of cytokines, including interferon-γ (IFNγ; Trinchieri, 1989; Seaman, 2000). Endometrial stromal cells produce a key effector of NK cell differentiation, interleukin-15 (IL-15; Ye et al., 1996; Dunn et al., 2002). It is logical to assume that trophoblast cells also influence uterine NK cell trafficking. The synthesis of PLP-A by trophoblast cells spatially and temporally coincides with the expansion, differentiation, and subsequent migration of uterine NK cells away from the developing chorioallantoic placenta (Campbell et al., 1989; Müller et al., 1999). PLP-A specifically binds to NK cells and also inhibits in vitro NK cell killing activity (Müller et al., 1999). In this report, we investigate PLP-A as a mediator of trophoblast cell modulation of uterine NK cells.

2. Materials and methods

2.1. Animals and tissue preparation

Holtzman rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). To obtain pregnant rats, females were caged overnight with fertile males. The presence of spermatozoa in vaginal smears was designated as day 0 of pregnancy. Conceptuses, decidual and placental tissues were dissected from pregnant animals (Soares, 1987; Roby et al., 1993). Tissues were frozen in liquid nitrogen for Western and Northern blot analysis. In situ PLP-A binding and immunocytochemistry were performed on tissues frozen in heptane pre-chilled on dry ice. All tissue preparations were stored at −80 °C until used. The University of Kansas Medical Center Institutional Animal Care and Use committee approved all procedures for handling and experimentation on animals.

2.2. In situ PLP-A binding assay

PLP-A interactions with targets were evaluated using an alkaline phosphastase (AP)-PLP-A fusion protein (Müller et al., 1999). In situ AP-PLP-A binding to tissues and cells were conducted as described previously (Müller et al., 1999). Competition experiments were performed with recombinant PLP-A (Deb et al., 1993), interleukin-2 (IL-2; R& D Systems, Minneapolis, MN), interleukin-7 (IL-7; R&D Systems), interleukin-12 (IL-12; R&D Systems), and IL-15 (R&D Systems).

2.3. Immunocytochemistry

Immunostaining was performed on cytospins of cells or on cryosections (10 μm) of tissues. A rabbit polyclonal anti-rat perforin antibody (Torrey Pines Biolabs, Houston, TX) was used at a concentration of 2.5 μg/ml to detect NK cells using a Histostain-SP kit (Zymed laboratories, San Francisco, CA). Identity of NK cells was further demonstrated by indirect immunofluorescence staining with a monoclonal ANK-61 antibody (Antibodies for Research BV, Doornenburg, The Netherlands), which recognizes a cell surface NK cell-specific antigen (Giezeman-Smits et al., 1997). Non-specific and isotype-matched control immunoglobulins were used as negative controls. Cells/tissues were fixed in ice-cold acetone for 10 min, incubated with 10% normal rat serum in phosphate buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH 7.4), for 15 min and then exposed for 1 h to ANK-61 antibodies. Samples were washed with several changes of PBS for 10 min, incubated for 30 min with FITC-conjugated rat anti-mouse IgG (Serotec Inc., Raleigh, NC) and rinsed in PBS. Samples were examined and images were recorded with a Nikon (Garden City, NY) phase/epifluorescence microscope equipped with appropriate filters and a CCD camera (Magnafire, Optronics, Goleta, CA).

2.4. NK cell culture systems

Uterine NK cells were isolated from mesometrial decidual tissue of day 10 pregnant rats by immunomagnetic cell sorting using a monoclonal antibody to gp42, a specific cell surface marker for activated NK cells (Imboden et al., 1989; Seaman et al., 1991), according to a previously described protocol (Müller et al., 1999). Purity was assessed by immunocytochemistry for perforin (see below) and cell viability was determined by trypan-blue exclusion (Hay, 1992). Uterine NK cells

were either centrifuged onto glass slides and processed for further characterization or used for culture. Freshly isolated NK cells were cultured at a concentration of 4×10^6 cells/ml for 10 h in RPMI 1640 medium supplemented with 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin and 10% fetal bovine serum (FBS) in the absence or presence of PLP-A (Deb et al., 1993) or IL-15 (R&D Systems). Recombinant PLP-A was purified from condition medium of CHO cells stably transfected with PLP-A expression vector as described previously (Deb et al., 1993).

Some aspects of NK cell biology were evaluated in the rat NK cell line, RNK-16 (Reynolds et al., 1984; Axberg et al., 1988). RNK-16 cells were maintained in RPMI 1640 culture medium supplemented with 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FBS.

2.5. Western blot analysis

Tissues were homogenized in a Tris saline buffer (50 mM Tris, 150 mM NaCl, pH 7.4) containing 1% NP-40, 1 mM EDTA, 0.1 mg/ml PMSF, and a protease inhibitor cocktail (Sigma Chemical Company, St. Louis, MO). Samples were then centrifuged and supernatants were collected. Protein concentrations were determined for each sample using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA). Proteins were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) under reducing conditions and were then transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH). The blots were then incubated for 2 h in blocking solution (5% nonfat dry milk in Tris buffered saline containing 0.1% Tween 20, TBST) and then overnight with a rabbit polyclonal anti-rat perforin antibody (Torrey Pines Biolabs, Houston, TX) diluted to 1:5000 in a blocking solution. IL-15 receptor α (IL-15Rα) was detected with a rabbit polyclonal antibody to IL-15Rα (sc9172, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and used at a 1:500 dilution. After washing the membranes in TBST, the blots were incubated with HRP-conjugated goat anti-rabbit IgG diluted 1:5000 in TBST for 1 h at room temperature. After washing with TBST, bound antibodies were detected by chemiluminescence following the manufacturer's instructions (ECL kit; Amersham Pharmacia Biotech, Piscataway, NJ).

2.6. Intracellular calcium measurement

Relative intracellular calcium concentrations in uterine NK cells exposed to PLP-A or IL-15 were measured using two different methods: image analysis and flow cytometry.

2.6.1. Image analysis

Freshly isolated uterine NK cells were loaded with Fluo-4AM (Molecular probes; Eugene, Oregon) in HBSS containing 0.5% BSA for 1 h at 25 °C. Cells were washed in HBSS containing 0.5% BSA to remove residual Fluo-4AM loading solution and then resuspended in the same buffer and incubated at 37 °C for 30 min for intracellular de-esterfication of Fluo-4AM. Cells were then analyzed in the absence and following treatment with PLP-A (1 μg/ml; Deb et al., 1993) or IL-15 (200 ng/ml; R&D Systems). Incubations and monitoring was performed on the stage of a Nikon inverted microscope equipped with a CCD camera. Fluorescent images were recorded every 5 min for a duration of 30 min and relative fluorescence intensity of cells was estimated using image analysis software Optimas™ 5.0 (Media Cybernatics, Bothell, WA).

2.6.2. Flow cytometry

Freshly isolated uterine NK cells were loaded with Fluo-4AM and treated with cytokines as described above. Cells were then analyzed using flow cytometry as described previously (Gee et al., 2000).

2.7. Measurement of IFNγ

IFNγ was measured using an ELISA kit from Biosource International (Camarillo, CA). Each sample was measured in duplicate and three different assays were performed using samples from three different experiments. Interassay coefficient of variation was between 3.4 and 5.5%. IFNγ content of each sample was normalized to the protein content of the sample.

2.8. Northern blot analysis

Northern blot analysis was performed as described previously (Faria et al., 1990). Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). Twenty micrograms of total RNA was resolved on 1% formaldehyde-agarose gels and transferred to nylon membranes followed by crosslinking. Blots were probed with a [αP^{32}] labeled cDNA for PLP-A. Glyceraldehyde-3′-phosphate dehydrogenase (G3PDH) cDNA was used to ensure the integrity and equal loading of RNA samples. At least three different tissue samples from three different animals were analyzed for each time point.

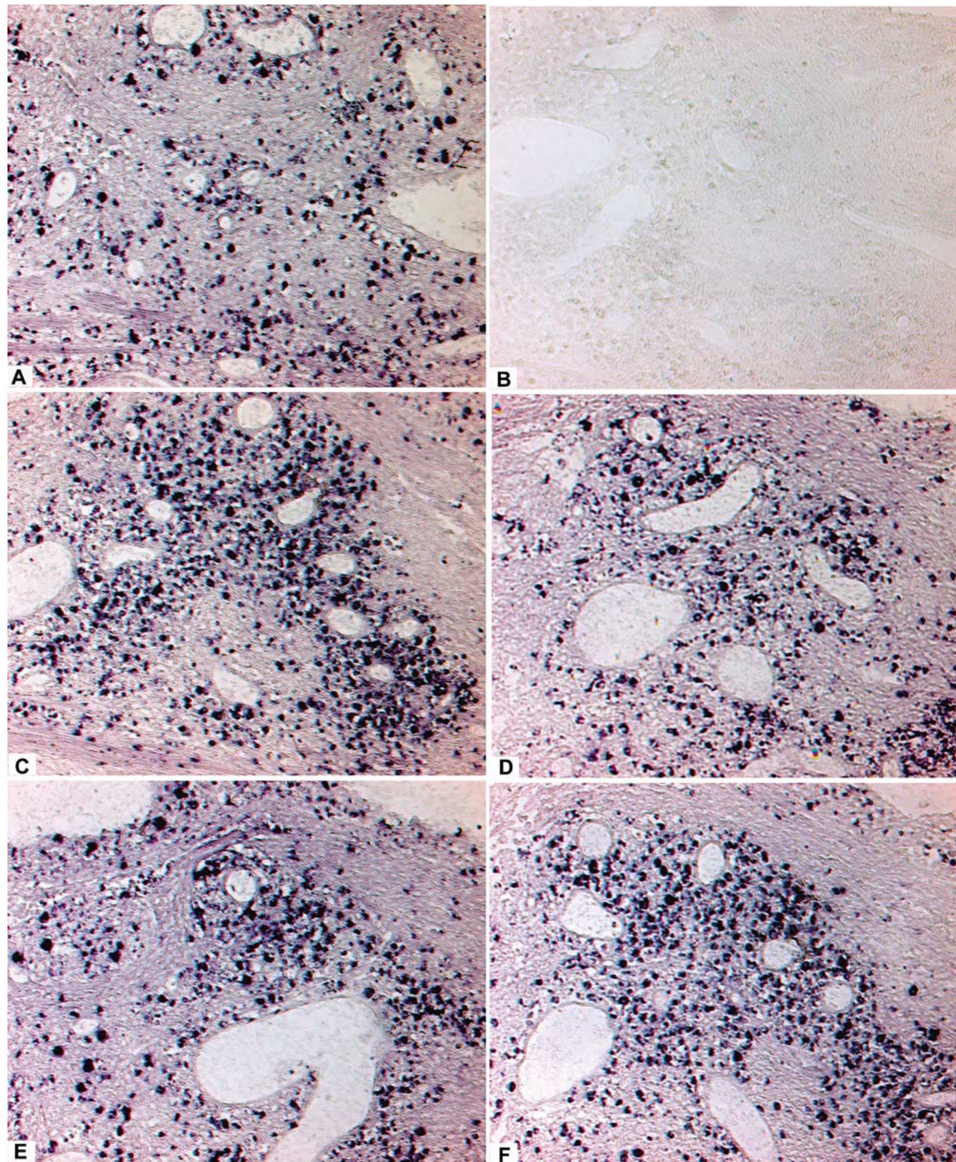

Fig. 1. PLP-A has a unique receptor on uterine NK cells. AP-PLP-A was used as a probe to assess the nature PLP-A interactions with NK cells in gestation day 14 metrial gland tissue sections. The ability of known NK cell ligands to compete for PLP-A binding to NK cells was evaluated. Panel A: AP-PLP-A; panel B: AP-PLP-A + PLP-A; panel C: AP-PLP-A + IL-2; panel D: AP-PLP-A + IL-7; panel E: AP-PLP-A + IL-12; panel F: AP-PLP-A + IL-15. For competition, PLP-A was used at 1 μg/ml, whereas other cytokines were added at concentrations of 10 μg/ml. Magnification 100 ×.

2.9. Statistical analysis

The data were analyzed by analysis of variance. The source of variation from significant F ratios was determined with Student's t-test (Keppel, 1973).

3. Results

3.1. Interactions of PLP-A with metrial gland NK cells

The gestation day-14 rat metrial gland is a rich source of NK cells and PLP-A binding activity (Müller et al., 1999). The nature of PLP-A binding to NK cells was evaluated by in situ binding of AP-PLP-A and competition with cytokines known to interact with NK cells (Fig. 1). AP-PLP-A binding to NK cells was effectively competed by PLP-A but was not competed by IL-2, IL-7, IL-12, or IL-15 (Fig. 1).

3.2. Characterization of isolated decidual NK cells

NK cells were isolated from gestation day-10 mesometrial decidual tissues using an immunomagnetic cell separation strategy with a monoclonal antibody to gp42. The monoclonal antibody to gp42 facilitated the enrichment of NK cells, as determined by immunocytochemical analyses for NK cell-specific proteins, perforin

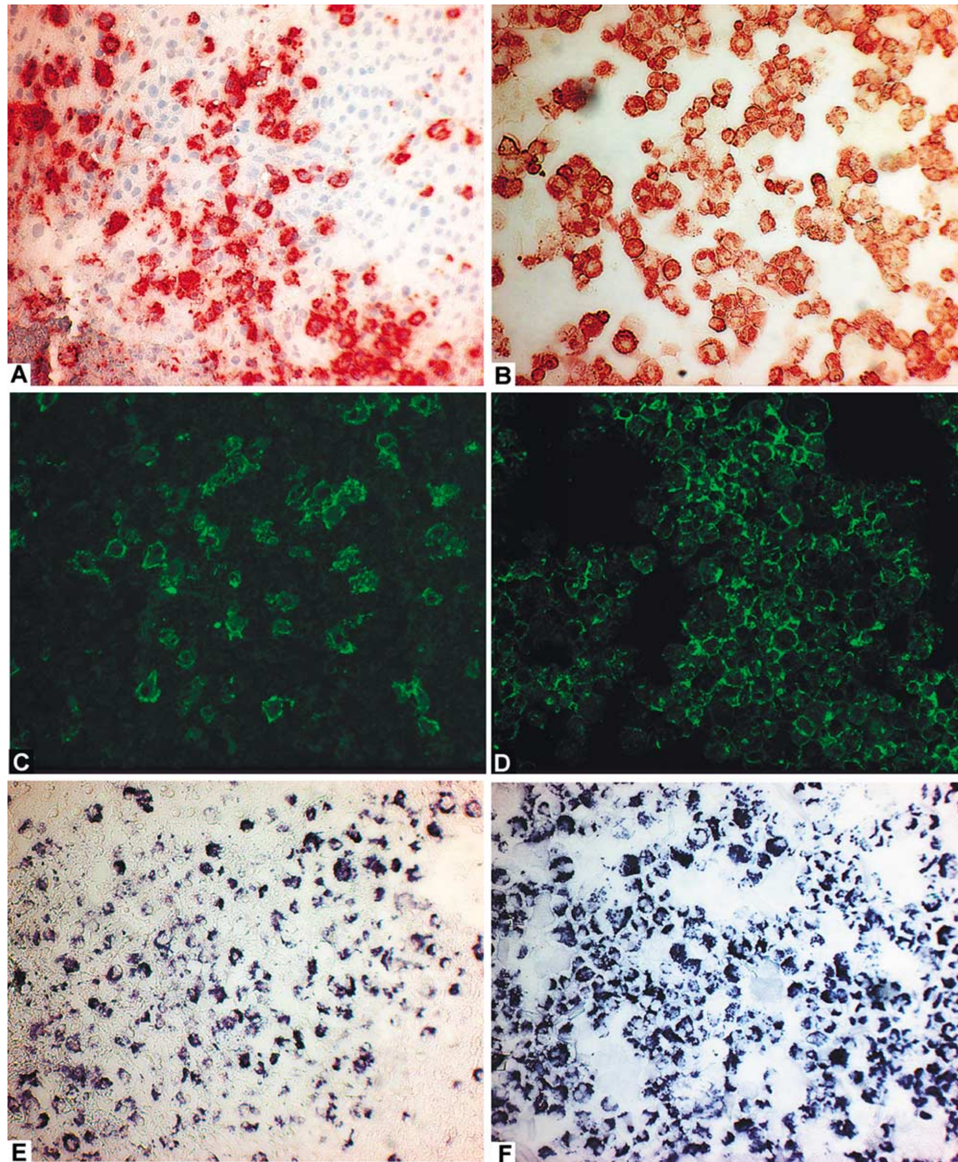

Fig. 2. Characterization of isolated uterine NK cells. NK cells were isolated from gestation day-10 uterine decidua using an immunomagnetic protocol with antibodies to gp42. Immunoisolated NK cells were characterized for expression of NK cell-specific proteins and PLP-A binding and compared to NK cells located in tissue sections. Panel A: immunolocalization of perforin in gestation day-14 metrial gland tissue; panel B: immunolocalization of perforin in isolated NK cells; panel C: immunolocalization of ANK-61 in gestation day-14 metrial gland tissue; panel D: immunolocalization of ANK-61 in isolated NK cells; panel E: AP-PLP-A binding to gestation day-14 metrial gland tissue; panel F: AP-PLP-A binding to isolated NK cells. Magnification 400 ×.

(Figs. 2A,B and 3), and ANK-61 antigen (Fig. 2C,D). Staining with nonspecific and isotype-matched control immunoglobulins was negative (data not shown). The purity of the NK cells was $>95\%$. These immunoisolated cells also bound to AP-PLP-A (Fig. 2E,F). AP-PLP-A bound to $>90\%$ of gp42-positive NK cells. Isolated decidual NK cells also expressed IL-15Rα protein (Fig. 3). RNK-16 cells, a rat NK cell line, also expressed perforin but not the IL-15Rα (Fig. 3). Viability of the recovered decidual NK cells was $>99\%$ at the time of isolation; $>99\%$ after 6 h of culture; $>85\%$ after 10 h of culture; $>50\%$ following 24 h of culture. The immunoisolated decidual NK cells were used to study the actions of PLP-A.

3.3. Effects of PLP-A on NK cell calcium

Cytokines can influence cellular activity via alteration of intracellular calcium concentrations ($[Ca^{2+}]_i$). Intracellular variations in $[Ca^{2+}]$ regulate many secretory events, and in immune cells, this regulated secretion provides a tight control over the delivery of highly bioactive effector proteins, thus representing a major mechanism of control of immune responses (Gardella et

al., 2001). PLP-A and IL-15 both elicited an increase in the $[Ca^{2+}]_i$ of a subpopulation of NK cells (Figs. 4 and 5). Three concentrations of PLP-A (0.5, 1, and 5 μg/ml) were evaluated. Maximal responses were achieved with a concentration of PLP-A at 1 μg/ml. IL-15 was used at a concentration of 200 ng/ml, a previously determined effective dose (Joshi et al., 2001). This was revealed by Fluo-4 imaging (Fig. 4) and by monitoring Fluo-4 by flow cytometry (Fig. 5). Results obtained using flow cytometry demonstrated that 20-25% of the total NK cell population responded to PLP-A or IL-15 with a sustained increase in $[Ca^{2+}]_i$. This intracellular calcium rise was mainly due to extracellular calcium influx, as it was completely abolished by the addition of the calcium chelator EGTA to the medium (Fig. 5).

3.4. *PLP-A functions as a mediator of trophoblast-NK cell signaling*

Uterine NK cells are a rich source of the multi-functional cytokine, IFNγ at the implantation site (Ashkar and Croy, 1999). Many of the functions of NK cells, including uterine vascular remodeling, are mediated by IFNγ (Ashkar and Croy, 2001). There is a paucity of information about the regulation of IFNγ production by NK cells. Thus we examined the effect of PLP-A on the ability of NK cells to produce IFNγ. PLP-A significantly inhibited IFNγ secretion by cultured NK cells within a 10 h time period (Fig. 6). In contrast, IL-15 stimulated IFNγ production by the cultured uterine NK cells (Fig. 6). Three concentrations of PLP-A (0.5, 1, and 5 μg/ml) were evaluated. The most effective concentration of PLP-A on IFNγ production was 5 μg/ml. IL-15 was used at a concentration of 200 ng/ml, a previously determined effective dose (Joshi et al., 2001). The inhibitory effect of PLP-A on NK cell IFNγ production is physiologically meaningful. In vivo, increased expression of PLP-A mRNA by the placenta during gestation coincides with a rapid decline of IFNγ production by NK cells located in the mesometrial decidua (Fig. 7). During the same gestational phase, mesometrial decidual NK cells showed increased production of perforin (Fig. 7). NK cells in the metrial gland, situated further away from the chorioallantoic placenta, expressed high levels of perforin and produced high levels of IFNγ (Fig. 7). Thus PLP-A is potentially a physiologically relevant regulator of NK cell IFNγ production.

4. Discussion

NK cells residing in the uterus contribute to successful pregnancy (Croy et al., 1998; Moffett-King, 2002). During gestation in the rat, uterine NK cells proceed through expansion, differentiation, migration, and death phases. The regulation of these events is not well understood. The known NK cell modulator, IL-15, has been implicated as a potential regulator of the expansion and differentiation phases of uterine NK cells (Ye et al., 1996). The origin of uterine IL-15 in the mouse is proposed to be macrophages infiltrating the uterine decidua (Ye et al., 1996). In the human, IL-15 and PRL produced by the decidual stroma have been shown to modulate the proliferation and differentiation of uterine NK cells (Dunn et al., 2002; Gubbay et al., 2002). Based on physical proximity, it is logical that trophoblast cells of the chorioallantoic placenta may orchestrate at least some aspects of NK cell development and trafficking. In this report, we show that PLP-A, a secretory product of trophoblast cells, specifically binds and modulates uterine NK cell function.

PLP-A specifically binds to uterine NK cells. Uterine cells binding PLP-A express the pore-forming protein, perforin, the activated NK cell surface antigens, ANK-61 and gp42, and the IL-15Rα subunit. Receptors for the related ligand, PRL, have been identified on NK cells and PRL has been shown to modulate NK cell function (Chambers et al., 1995; Matera and Mori, 2000). PLP-A is considered a nonclassical member of the PRL family and does not utilize the PRL receptor (Deb et al., 1993; Müller et al., 1999). NK cell surface molecules mediating PLP-A interactions do not mediate the interaction of NK cells with other cytokines known to modulate NK cell function, including IL-2, IL-7, IL-12, and IL-15 (Deb et al., 1993; Müller et al., 1999, present study). However, this result does not imply that the PLP-A-NK cell interaction is unique. PLP-A may utilize a ligand–receptor system not investigated in the present study.

Uterine NK cell signaling was affected by PLP-A and IL-15. Both cytokines stimulated intracellular calcium mobilization. Although PLP-A bound most uterine NK cells, only a subpopulation of cells responded to PLP-A

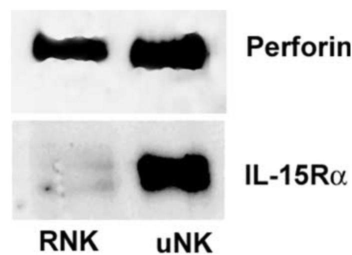

Fig. 3. Uterine NK cells express the IL-15 receptor alpha protein. Western blot analyses of perforin and IL-15Rα protein expression in the RNK-16 NK cell line and in isolated uterine NK cells. Total cellular proteins were fractionated by SDS-polyacrylamide gel electrophoresis in 10% polyacrylamide gels. Proteins were transferred to nitrocellulose membranes and probed with perforin or IL-15 Rα antibodies. Please note that though both RNK-16 rat NK cells and uterine NK cells possess comparable amounts of perforin, however, uterine NK cells have substantially more IL-15Rα than present in the RNK-16 cell line.

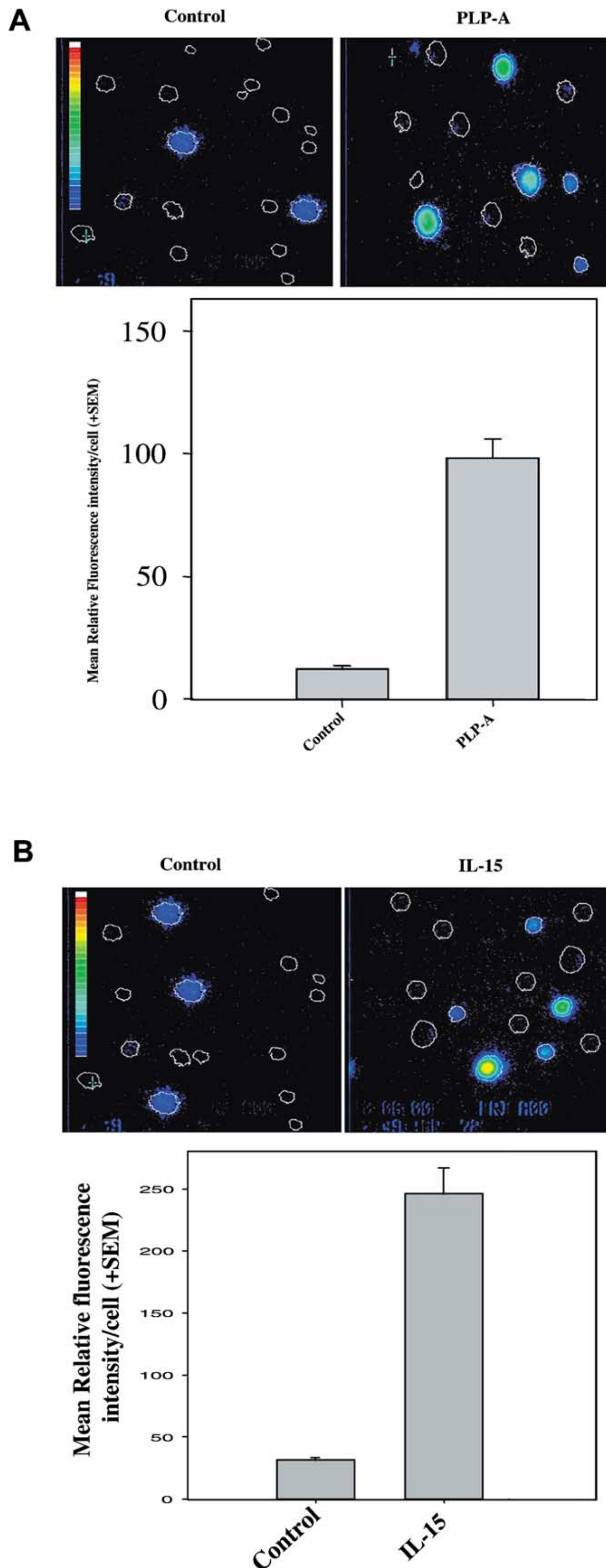

Fig. 4

Fig. 4. Influence of PLP-A on total intracellular Ca^{+2} in uterine NK cells. Changes in intracellular Ca^{+2} in uterine NK cells were monitored by loading cells with Fluo-4AM followed by fluorescence imaging. All intracellular Ca^{2+} responses were determined after autozeroing basal (unstimulated) level. Panel A: pseudocolor digitized cellular images are shown in control or after stimulation of uterine NK cells with PLP-A (1 μg/ml; upper panel). Mean relative fluorescence intensity per cell was calculated using image analysis software Optimas™ 5.0 (lower panel). Panel B: pseudocolor digitized cellular images are shown in control or after stimulation of uterine NK cells with Il-15 (200 ng/ml; upper panel). Mean relative fluorescence intensity per cell was calculated using image analysis software Optimas™ 5.0 (lower panel).

with changes in intracellular calcium mobilization. IL-15 actions on intracellular calcium were similarly restricted to a subpopulation of NK cells. These results suggest that within the implantation site NK cells might exist at various stages of differentiation. Cytoplasmic calcium increases are important signals in nonexcitable cells, including immune cells, and regulate fundamental processes such as activation, growth, and differentiation (Gardner, 1989; Clapham, 1995). IL-15 promotes NK cell development, survival, and host responses to pathogens (Waldmann and Tagaya, 1999). Presumably some of these IL-15 actions are mediated by intracellular calcium mobilization. PLP-A also stimulated NK cell calcium mobilization but did not mimic other effects of IL-15 on uterine NK cells (present study).

PLP-A and IL-15 possessed opposing actions on uterine NK cell release of IFNγ. During pregnancy, IFNγ is proposed to be a key mediator of NK cell actions on the uterine mesometrial vasculature (Ashkar and Croy, 2001). Thus, IFNγ potentially impacts the flow of nutrients to the placenta and fetus. PLP-A inhibited uterine NK cell IFNγ production, while IL-15 stimulated uterine IFNγ production (present study). Interestingly, PRL is capable of synergizing with IL-2 or IL-12 in stimulating NK cell IFNγ production (Matera and Mori, 2000). The presence of ligands for the PRL receptor within the implantation site (placental lactogen-I and placental lactogen-II, Faria et al., 1990) could promote IFNγ production if not opposed by the presence of PLP-A. Concomitant with increased production of PLP-A by the chorioallantoic placenta, there is a rapid decrease in IFNγ content in the mesometrial decidua adjacent to the developing chorioallantoic placenta. Based on our in vitro data, we would predict that the decline of decidual NK cell IFNγ is directed by PLP-A. Furthermore, the decline in decidual IFNγ is likely an essential factor permitting continued placental development. In support of this hypothesis, IFNγ is known to inhibit trophoblast cell outgrowth from blastocysts (Haimovici et al., 1991; Ain et al., 2003) and in the human can inhibit decidual cell PRL production (Christian et al., 2001). Hence the timing of placental PLP-A expression and its actions on NK

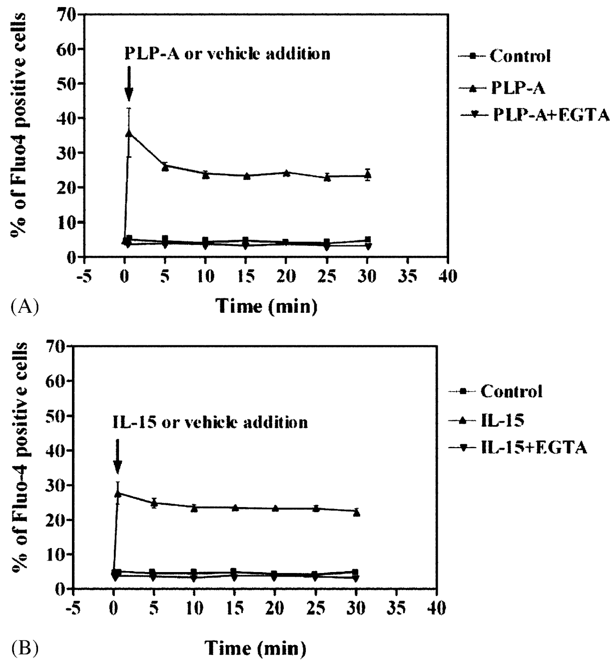

Fig. 5. PLP-A stimulates Ca^{+2} influx in uterine NK cells. Time dependent changes in uterine NK cell intracellular $[Ca^{+2}]$ were monitored by loading cells with Fluo-4AM followed by flow cytometry performed at five min intervals. All intracellular $[Ca^{2+}]$ responses were normalized to the basal (untreated) condition. Panel A: treatment with either PLP-A (1 μg/ml) or PLP-A (1 μg/ml)+2 mM EGTA. Panel B: treatment with either IL-15 (200 ng/ml) or IL-15+2 mM EGTA.

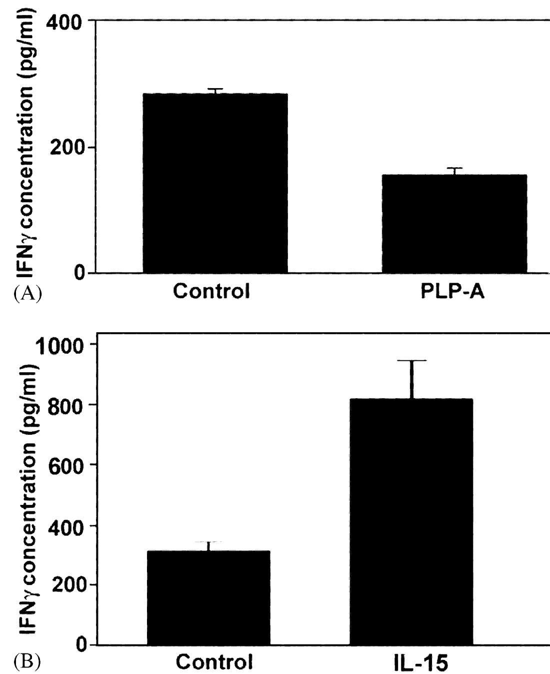

Fig. 6. PLP-A inhibits and IL-15 stimulates secretion of IFNγ by uterine NK cells. Freshly isolated uterine NK cells were cultured for 10 h at a concentration of 4×10^6/ml in the absence or presence of PLP-A (5 μg/ml; optimized concentration, top panel) or IL-15 (200 ng/ml, bottom panel). Conditioned medium was collected and IFNγ was measured by ELISA. Data represents the results from four replicate experiments. Control vs. PLP-A or IL-15, $P < 0.05$.

cell IFNγ production appear linked and beneficial for the maintenance of pregnancy. The impact of PLP-A on uterine NK cells is selective. While increased PLP-A was associated with declining decidual NK cell IFNγ, decidual NK cell perforin content increased. Metrial gland NK cells situated more distally from the chorioallantoic placenta exhibited high concentrations of IFNγ. These findings suggest that PLP-A's actions on NK cells do not mimic the actions of other NK cell activators and likely are of a paracrine nature. The association of PLP-A with circulating PLP-A binding proteins likely obviates the effects of PLP-A on distant sites (Deb et al., 1993). The metrial gland is comprised of a large collection of blood vessels supplying the placenta and the fetus. Continued IFNγ production by NK cells within the metrial gland after midgestation may be essential for additional uterine vascular remodeling necessary for optimal fetal growth, as proposed by Ashkar and Croy (2001).

During a normal pregnancy, uterine NK cells expand in numbers and differentiate but do not acquire a classic activated NK cell phenotype (Croy et al., 1998; Moffett-King, 2002). The genetically disparate embryonic and extraembryonic tissues would appear to be a sufficient stimulus for activation of NK cell killing. However, uterine NK cells of pregnancy show a limited capacity for killing. Considerable experimentation in the human has implicated histocompatibility antigen-NK cell inhibitory receptor signaling in the inhibition of NK cell killing activities during pregnancy (Moffett-King, 2002). Prostaglandins have also been implicated in mediating pregnancy-associated changes in the uterine NK cell phenotype (Lala, 1989; Lala et al., 1990; Linnemeyer and Pollack, 1993). In this report, PLP-A has been shown to mediate signaling from trophoblast cells to uterine NK cells. We propose that PLP-A contributes to modulating the uterine NK cell phenotype of pregnancy.

Finally, given the prominence of NK cells in the uterus during pregnancy in rodents and the human, it is likely that functional homologies exist across species regarding trophoblast-NK cell signaling. Attempts at identification of a human homologue for PLP-A from DNA/protein database searches have not proven successful. It is, however, important to appreciate that lack of overall structural identity does not preclude similarities in biological functions. It seems logical that PLP-A may share a functional domain(s) with other NK cell regulators. The validity of this prediction will require identification of the NK cell surface signaling molecules activated by PLP-A.

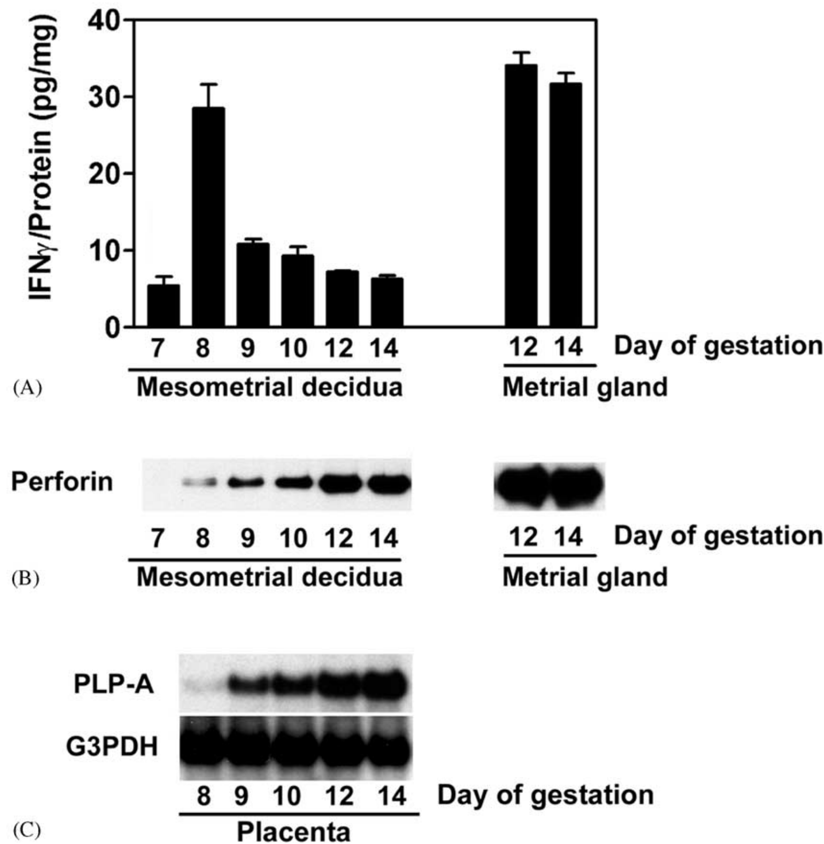

Fig. 7. Temporal patterns of placental PLP-A expression and mesometrially expressed IFNγ. Panel A: IFNγ concentrations were measured in extracts of freshly dissected mesometrial decidual tissue and metrial gland tissue from different days of pregnancy. IFNγ concentrations were measured by ELISA and normalized to protein content. Data presented are means $\pm$ standard error of the mean of triplicate experiments. Panel B: Western blot analyses for perforin in extracts of freshly dissected mesometrial decidual tissue or metrial gland tissue from different days of pregnancy. Equal amounts of protein from different samples were fractionated by in 10% polyacrylamide gels. Proteins were transferred to a nitrocellulose membrane and probed with antibodies to perforin. Panel C: placental PLP-A mRNA concentrations were estimated by Northern blot analyses. Total RNA was isolated from the junctional zone of the rat chorioallantoic placenta on gestational days 8–14, fractionated by formaldehyde-agarose gel electrophoresis, transferred to nylon, and hybridized to a [^{32}P]-labeled cDNA probe for PLP-A. Consistent gel loading was verified by monitoring G3PDH expression.

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