Localization of Placental Lactogen-I in Trophoblast Giant Cells of the Mouse Placenta

TERESA N. FARIA, LINDA OGREN, FRANK TALAMANTES, DANIEL I. H. LINZER, and MICHAEL J. SOARES

Department of Physiology, University of Kansas Medical Center, Kansas City, Kansas 66103
Department of Biology, Sinsheimer Laboratories, University of California, Santa Cruz, California 95064
Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University
Evanston, Illinois 60201

ABSTRACT
The purpose of this investigation was to identify the cellular origin of placental lactogen-I (PL-I) expression in the mouse placenta and to cytologically define the transition from PL-I to PL-II expression during gestation. PL-I mRNA expression was assessed by in situ hybridization, and expression of PL-I and PL-II protein was determined by immunocytochemical analysis. PL-I mRNA and protein were localized to trophoblast giant cells. Trophoblast giant cells ceased producing PL-I at midgestation and began expressing PL-II. PL-I immunoreactivity was present in trophoblast giant cells on Days 9 and 10 of gestation but was not detectable in trophoblast giant cells on Day 11 of gestation. Immunoreactive PL-II-producing giant cells were detected first on Day 10 of gestation, continuing on Day 11 of gestation. Expression of PL-I and PL-II signals a significant functional transition in trophoblast giant cells of the developing mouse placenta.

INTRODUCTION
The mouse placenta is a source of a number of proteins related to pituitary prolactin (see Ogren and Talamantes [1], for a review). Placental lactogen-I (PL-I) is the first of these prolactin-related proteins expressed during gestation [2]. PL-I expression is initiated shortly after implantation and terminates at midgestation [2]. Placental lactogen-II (PL-II) is the second placental prolactin expressed during gestation. PL-II production is initiated at midgestation and continues until term [3]. Both PL-I and PL-II are potent functional analogues of pituitary prolactin [4, 5].

To gain insight into the control of PL expression in the developing mouse placenta, we have investigated the placental cell types responsible for expressing PL-I and PL-II. Previous studies have shown that PL-II expression is restricted to trophoblast giant cells [6, 7]. In this report, we confirm the localization of PL-II to trophoblast giant cells and show that PL-I protein and mRNA are also expressed by trophoblast giant cells. Although, PL-I and PL-II are expressed by the same cell type, each hormone shows a unique temporal pattern of expression.

MATERIALS AND METHODS

Reagents
Nitrocellulose was obtained from Schleicher and Schuell Inc. (Keene, NH). Restriction enzymes and polymerases were purchased from New England Biolabs (Beverly, MA). Reagents used for the synthesis of complementary RNA probes, DNase-I, and dextran sulfate were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Radiolabeled nucleotides were obtained from DuPont-NEN (Boston, MA). Avidin-Biotin immunoperoxidase kits for rabbit immunoglobulin G (IgG) were purchased from Vector Laboratories (Burlingame, CA). Unless otherwise noted, all other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and Tissue Preparation
Swiss-Webster mice were obtained from Simonsen Laboratories (Gilroy, CA). Timed pregnancies were generated by housing females with males and daily inspection for copulatory plugs. The day a copulatory plug was detected was designated Day 0 of pregnancy. Conceptuses were removed from uteri during midgestation (Days 9 through 11), fixed in paraformaldehyde (4%, w/vol in PBS; 10 mM sodium phosphate, pH 7.2; and 150 mM NaCl) and used for in situ hybridization analysis or fixed in freshly prepared Bouin’s fluid and used for immunocytochemical analysis.

Immunocytochemistry
Placental tissues fixed in Bouin’s fluid were dehydrated, cleared, embedded in paraffin, and sectioned at 7 µm. Placental tissue was immunocytochemically stained for the presence of either PL-I or PL-II by use of an avidin-biotin immunoperoxidase kit for rabbit IgG as previously described [8]. PL-I expression was assessed with an antiserum to recombinant mouse PL-I [5], and PL-II expression was assessed with an antiserum to amino acids 56–70 of rat PL-II [9]. The PL-I and PL-II antisera were both used at a 1:200 dilution. The immunostained tissues were counterstained
with hematoxylin. Control sections were examined by using antisera saturated with antigen.

**In Situ Hybridization**

PL-I mRNA was detected in tissue sections as previously described [10]. The PL-I cDNA [11] subcloned into a pGEM plasmid was used as a template for the synthesis of $[^{35}S]$-

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**FIG. 1.** Immunocytochemical analysis of PL-I and PL-II within sections from conceptuses isolated on Day 9 of gestation. The sections were stained for the presence of PL-I using an avidin-biotin immunoperoxidase kit for rabbit IgG. PL-I was detected with a rabbit antiserum to recombinant mouse PL-I (a). Control sections were stained with antiserum to PL-II (b). Note the intense staining of the trophoblast giant cells in a (arrows). $\times 40$.

**FIG. 2.** Immunocytochemical analysis of PL-I and PL-II within sections of conceptuses from Days 9 through 11 of gestation. (a) and (d), Day 9 conceptus; (b) and (e), Day 10 conceptus; (c) and (f), Day 11 conceptus. (a–c), Stained for PL-I; (d–f), stained for PL-II. Each micrograph shows a portion of the developing placenta containing a number of representative trophoblast giant cells. Note the shift from PL-I immunostaining to PL-II immunostaining after Day 10 of pregnancy. a, b, d, and e: $\times 100$; c and f: $\times 200$. See Figure 1 and the text for further information regarding methodology.
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FIG. 3. Cellular localization of PL-I mRNA within conceptuses on Day 10 of gestation. PL-I mRNA was hybridized in situ for 4 h at 42°C with [35S]-labeled PL-I antisense (a and c) and sense (d) probes. Photographs show a portion of representative trophoblast giant cells. Note the intense hybridization with the PL-I antisense RNA probe in (c) and the absence of hybridization with the PL-I sense RNA probe in (d). Dec, decidua.
labeled antisense and sense RNA probes. The RNA probes used in the analysis were derived from an 800-base pair cDNA representing the entire coding region of the PL-I mRNA.

RESULTS AND DISCUSSION

PL-I protein and mRNA were specifically localized to trophoblast giant cells of the mouse placenta (Figs. 1, 2, and 3). The specificity of the localization was determined by competition with excess peptide, hybridization with sense RNA probes, and the differential distribution of PL-I and PL-II within the mouse conceptus. These findings are consistent with the localization of PL-I to trophoblast giant cells of the rat placenta [10]. PL-II was also localized to mouse trophoblast giant cells (Fig. 2), consistent with previous reports [6, 7].

Specific temporal factors appear to be involved in the control of trophoblast giant cell PL-I expression. PL-I expression is initiated shortly after blastocyst implantation [2, 10]. A significant developmental event occurs between Days 9 and 11 of gestation. The event is marked by the initial detection of PL-II immunoreactive trophoblast giant cells on Day 10, followed by the termination of trophoblast giant cell PL-I expression on Day 11 (Fig. 2). Trophoblast giant cell expression of PL-II continues during the remainder of pregnancy [6]. The factors involved in controlling the cell-and temporal-specific patterns of PL-I and PL-II expression are yet to be determined. Trophoblast giant cell-specific expression of PL-I and PL-II may be dictated by the presence of common structural elements associated with genes for each hormone [12, 13].

The transition from PL-I to PL-II expression marks an important shift in the control of pregnancy from the anterior pituitary to the placenta [14, 15]. Expression of two functional analogues of pituitary prolactin, PL-I and PL-II, during this important developmental transition may permit more precise regulation of the availability of a prolactin-like hormone. Alternatively, each PL may possess actions specialized for developmental events occurring during its phase of expression. For example, PL-I may act to promote the growth and differentiation of uterine decidua and the corpus luteum [1, 16], whereas PL-II may act to maintain corpus luteum function, stimulate mammary gland development, and promote fetal growth [1]. There is a biochemical basis for these putative specialized actions of PL-I and PL-II. Biochemically, PL-I and PL-II have distinct differences in their amino acid sequences [17, 18] and in their post-translational modifications [18]. The addition of carbohydrate to PL-I has been speculated to be responsible for its longer circulating half-life [10, 18, 19]. A more precise explanation for the expression of multiple placental prolactin-like hormones during gestation awaits further experimentation.

In conclusion, expression of PL-I and PL-II signals a significant functional transition in trophoblast giant cells of the developing mouse placenta.

During the review of this manuscript a report appeared confirming the immunolocalization of mouse PL-I to trophoblast giant cells [20].

ACKNOWLEDGMENT

The authors would like to thank Linda Carr for assistance with the preparation of the manuscript.

REFERENCES