Induction of Cytotoxic T-lymphocyte Antigen-2β, A Cysteine Protease Inhibitor in Decidua

A POTENTIAL REGULATOR OF EMBRYO IMPLANTATION*

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During early pregnancy, the steroid hormone progesterone induces differentiation of uterine stroma to decidual cells, which regulate embryo-uterine interactions. The progesterone-induced signaling molecules that participate in the formation and function of decidua remain poorly understood. We recently utilized high-density oligonucleotide microarrays to identify several genes whose expression is markedly altered in pregnant uterus in response to RU486, a well characterized antagonist of the progesterone receptor (PR). Our study revealed that the gene encoding cytotoxic T-lymphocyte antigen-2β (CTLA-2β), a cysteine protease inhibitor, is expressed during PR-induced decidualization. The spatio-temporal expression of CTLA-2β mRNA precisely overlapped with the decidual phase of pregnancy. Interestingly, administration of progesterone to estrogen-primed ovariectomized mice failed to induce CTLA-2β expression. A concomitant artificial decidual stimulation was necessary to trigger this expression. Uteri of PR knockout mice failed to express this mRNA, even after a combined administration of steroid hormones and artificial stimulation. The uterine expression of CTLA-2β was, therefore, dependent on PR as well as other unknown factor(s) associated with decidual response. To identify the molecular target(s) of CTLA-2β, we analyzed its interaction with proteins present in soluble extracts prepared from day 7 pregnant uteri containing implanted embryos. A protein affinity strategy employing recombinant CTLA-2β helped us to determine that cathepsin L, a cysteine protease, is one of its targets in the pregnant uterus. Consistent with this finding, expression of cathepsin L was detected in the giant trophoblast cells of the ectoplacental cone on day 7 of pregnancy. Collectively, our results support the hypothesis that expression of CTLA-2β in the decidua may regulate implantation of the embryo by neutralizing the activities of one or more proteases generated by the proliferating trophoblast.

Progesterone plays a crucial role during early pregnancy by coordinating a complex series of interactions between the implanting blastocyst and the receptive uterus (1–5). In mice, implantation is initiated 4 days after fertilization when the blastocyst reaches the uterus (1, 6). The hormonal regimen necessary to prepare a non-receptive uterus to receive embryo and permit implantation involves 48 h of progesterone treatment followed by a dose of estrogen (1, 7–9). Whereas the combined action of estrogen and progesterone is essential for acquisition of uterine receptivity, progesterone is the critical hormone for decidualization (1, 10). Decidualization involves differentiation of the fibroblast cells of endometrial stroma into morphologically distinct cells, termed decidual cells, which show unique secretory and biosynthetic properties (10, 11). This differentiation, induced by progesterone following a brief priming by estrogen, is a prerequisite for successful implantation. Decidua is a transient tissue, which first develops at the time of blastocyst attachment on day 4 of pregnancy. During the next 3 days, the decidual cells proliferate and differentiate extensively, and eventually they undergo apoptosis. By the end of the invasive period (day 10.5), the decidua is regressed. A variety of functions have been attributed to the decidua (12–15). It facilitates nutrient transfer to the embryo and secretes hormones, growth factors, and cytokines that are critical for embryonic growth and development. Additionally, the two most widely accepted roles of decidua are regulation of trophoblast invasion and immunomodulation of fetal allograft during pregnancy (12–15). The progesterone-regulated factors that play critical roles during decidualization, leading to successful implantation and establishment of pregnancy, however, remain largely unknown.

To understand how progesterone regulates implantation, it is essential to identify a broader spectrum of genes that are regulated by PR in this process. To achieve this goal, we employed RU486, a well characterized antagonist of PR function during pregnancy (16, 17). RU486 counteracts PR-dependent pathways by binding to the receptor and impairing its gene regulatory function (16, 17). We used oligonucleotide microarrays to identify the genes whose uterine expression is markedly altered at the time of implantation by RU486-complexed PR (18). Here we report the identification of a gene encoding cytotoxic T-lymphocyte antigen-2β (CTLA-2β) that is induced downstream of PR in the pregnant uterus.

Denizot et al. (19) first cloned CTLA-2β from a T lymphocyte-derived cDNA library and found it to be highly homologous to the proregion of cysteine proteases. Previous studies indicated that the proregion, purified from a given protease, can inhibit the activity of that protease in vitro (20). Consistent with this

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§ The abbreviations used are: PR, progesterone receptor; CTLA-2β, cytotoxic T-lymphocyte antigen-2β; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DIG, digoxigenin.
paradigm, studies have shown that CTLA-2b is a competitive inhibitor of certain cysteine proteases including cathepsin B and L (20). We observed that CTLA-2b is induced in the steroid-primed uterus in response to a decidual signal. In situ hybridization analysis indicated that the expression of CTLA-2b in the pregnant uterus is localized in the uterine stroma near the vicinity of the embryo and overlapped with the decidual plug. Interestingly, our studies further revealed that cathepsin L, which is expressed in sertoli trophoblast cells, is one of the targets of CTLA-2b. Based on these studies, we postulate that expression of CTLA-2b in uterine stroma during PR-mediated decidualization plays a critical role in the regulation of embryo implantation.

**MATERIALS AND METHODS**

**Reagents**—Progesterone and 17β-estradiol were purchased from Sigma. RLU 38486 (Mifepristone) was a gift of Population Council, New York, NY. Antibody against cathepsin L was purchased from Santa Cruz Biotechnology, Santa Cruz, CA.

**Animals and Tissue Collection**—All experiments involving animals were approved by the Animal Care Committee at the University of Illinois, Urbana-Champaign, and the studies were conducted in accordance with the National Institutes of Health standards for the use and care of animals. Female mice (CD-1 from Charles River, Wilmington, MA), in proestrus, were mated with adult males. The presence of a vaginal plug after mating was designated as day 1 of pregnancy. The animals were killed at various stages of gestation and the uterus were collected. The PRKO mice were bred and homozygote were confirmed by genotyping as described previously (18).

**Artificial Decidualization—Decidualization was induced artificially as described previously (21). Mice were first ovarioctomized. Two weeks following ovarioctomy, animals were injected with 100 ng of estrogen in 0.1 ml of sesame oil for three consecutive days. This was followed by daily injections of 1 mg of progesterone for three consecutive days. Then one horn of the uterus was dramatically stimulated by insertion of a burred needle and longitudinal scratching of the entire length of the uterine horn. The other horn was left unstimulated. The animals were treated with progesterone for an additional 6 days post-stimulation and then sacrificed. The uteri were collected, the two horns were separated, and RNA was isolated from each horn.

**Northern Blot Analysis**—For Northern analysis, 20–30 μg of total RNA or 5 μg of poly(A)+RNA was separated by formaldehyde-agarose gel electrophoresis and transferred to Duralon membrane (Stratagene). Blots were prehybridized in 50 mM NaPO₄ pH 6.5, 5× SSC, 5× Denhardt’s, 50% formamide, 0.1% SDS, and 100 μg/ml salmon sperm DNA for 4 h at 42 °C. Hybridization was carried out overnight in the same buffer containing 10⁶ cpm/ml of a 32P-labeled CTLA-2b cDNA fragment. The filters were washed twice for 15 min in 1× SSC, 0.1% SDS at room temperature, then twice for 20 min in 0.2× SSC, 0.1% SDS at 55 °C and then exposed to x-ray films. The intensities of signals on the autoradiogram were estimated by densitometric scanning. To correct for RNA loading, the obtained signals were normalized with respect to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal in the same blot. For this the filters were stripped of the radioactive probe by washing for 10 min in 0.5% SDS at 95 °C. The blots were then re-probed with a 32P-labeled GAPDH probe as described above.

**In Situ Hybridization**—Uterine tissues from pregnant animals were collected and frozen. Tissues were fixed in 4% paraformaldehyde at 4 °C. Cryostat sections were cut at 8 μm and attached to 3-amino-1-propylthiathymine-diacrylamide (Tgna) coated slides. In situ hybridization was performed with digoxigenin (DIG)-labeled sense or antisense RNA probes complimentary to CTLA-2b gene. DIG-labeled RNA probes were synthesized from CTLA-2b cDNA using T3 or T7 RNA polymerase and DIG-labeled nucleotides according to the manufacturer's specifications (Roche Molecular Biochemicals). Prehybridization was carried out in a 50% formamide-containing hybridization buffer (50% formamide, 5× SSC, 2% blocking reagent, 0.02% SDS, 0.1% N-lauryl-sarcosine). Hybridization was carried out at 42 °C overnight in a damp humidified chamber. To develop the substrate, sections were sequentially washed in 2× SSC, 1× SSC, and 0.1× SSC for 15 min in each buffer at 37 °C. Sections were then incubated with anti-DIG alkaline phosphatase-conjugated antibody. Excess antibody was washed away and the color substrate (nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indoophosphate) was added. Slides were allowed to develop in the dark and the color was visualized under light microscopy until maximum levels of staining were achieved. The reaction was stopped and the slides were counterstained in Nuclear Fast Red for 5 min. The slides were washed in water, dehydrated, and covered slipped. Control incubations utilized a DIG-labeled RNA sense strand and were performed under identical conditions.

**Immunohistochemistry**—Polyclonal antibody against cathepsin L (Santa Cruz Biotechnology, Santa Cruz, CA) was diluted 1:1000 for immunohistochemistry. Frozen uteri were sectioned at 7 μm, mounted on slides, and then fixed in 5% formaldehyde in phosphate-buffered saline. Sections were washed in phosphate-buffered saline for 20 min and then incubated in a blocking solution containing 10% normal goat serum for 10 min before incubation in primary antibody overnight at 4 °C. Immunostaining was performed using a streptavidin-biotin kit for rabbit primary antibody (Zymed Laboratories Inc., Burlingame, CA). Deposition of immunostaining was verified by negative controls.

**Baculovirus Expression and Purification of CTLA-2b**—To express recombinant CTLA-2b in baculovirus, the leader sequence was removed and the serine residue at position 31 was changed to methionine. A fragment of CTLA-2b with NcoI and BamHI sites was generated by PCR and ligated into the NotI and BglII sites of a pAcGS2 vector (BD Pharmingen). At the N-terminal end of CTLA-2b, double stranded 3× FLAG oligo was inserted. The transfection of this vector into a baculovirus/insect cell system was performed following the manufacturer's protocol (Baculogold™ Transfection kit, BD Pharmingen).

SF9 insect cells were grown in Grace's insect medium/TNM-FH (In-vitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT). The N-terminal FLAG-tagged recombinant CTLA-2b was expressed in SF9 cells. Cells were infected with the recombinant virus at a multiplicity of infection of 10 to 1 at 27 °C. Cells were harvested at 96 h post-infection. After harvesting, the baculovirus cell culture supernatant was supplemented with a mixture of protease inhibitors (BD Pharmingen). The supernatant was then incubated with resin linked to a monoclonal antibody against FLAG (Sigma) for 3 h at 4 °C on a rocking platform. The resin was washed three times with 20 bed volumes of TBS buffer (50 mM Tris-CI, 150 mM NaCl, pH 7.4). The proteins bound to the resin were eluted at 4 °C with a buffer containing 0.2 mg/ml FLAG peptide in TBS. After elution, specific protein expression was confirmed by Western blotting using a FLAG antibody. The analysis of purified CTLA-2b was performed by SDS-PAGE and Coomassie staining. Protein concentration was determined by the Bradford method.

**RESULTS**

**Antiprogestin RU486 Down-regulates CTLA-2b Expression in the Pregnant Uterus**—The pivotal role of progesterone during early pregnancy is likely to be mediated by a network of PR-induced genes (22). Consistent with this hypothesis, treatment of pregnant rat or mouse during the pre-implantation phase with RU486, which impairs transcriptional function of PR, blocked implantation (23). To identify the downstream targets of PR during pregnancy, we have recently examined the global changes in uterine mRNA expression profiles in response to RU486 (18).

Briefly, mice on day 3 of pregnancy were treated with either vehicle (sesame oil) or RU486 and uterine tissues were collected on day 4, the day of implantation. The uterine transcripts were then hybridized to high-density oligonucleotide arrays (murine GeneChip Expression arrays, Affymetrix, Santa Clara, CA) containing ~12,000 gene probes. We applied a threshold of a 3-fold change in expression level between RU486-treated samples and untreated controls for identifying putative progesterone-regulated mRNAs. Applying this stringent cut off, we identified a total of 67 mRNAs whose expression was down-regulated in the uterus at the time of implantation in response to RU486 (18). We identified one of these genes as that encoding CTLA-2b, a cysteine protease inhibitor.

To verify the results of microarray analysis, we performed Northern blotting using total RNA obtained from uteri of day 4 pregnant mice treated with or without RU486 (Fig. 1). When the blot was probed with 32P-labeled CTLA-2b cDNA, a signal appeared in the RNA sample obtained from untreated mice on day 4 of pregnancy (lane 1). No mRNA signal was observed in the uterine sample obtained from RU486-treated mice (lane 2). Hybridization of the same blot with a control probe (GAPDH) indicated equal loading of mRNAs in these lanes. These re-
sults, therefore, confirmed the down-regulation of the level of CTLA-2β mRNA as predicted by the microarray analysis.

Profile of CTLA-2β Expression in Uterus during Early Pregnancy—We next investigated the pattern of CTLA-2β gene expression in the mouse uterus at various stages of gestation. We performed Northern blot analysis of RNA isolated from uteri of mice at days 1, 2, 3, 4, 5, 6, 8, and 10 of pregnancy (Fig. 2). Whole uteri devoid of embryos were used for this experiment. The uteri were freed of embryonic tissue either by repeated flushings (days 1–5) or by individually removing the embryos from the implantation sites (days 6–10). The blot was hybridized with a 32P-labeled CTLA-2β probe and also a control GAPDH probe. As depicted in Fig. 2A, no signal corresponding to CTLA-2β mRNA was observed in the pregnant animals on days 1–3 of gestation (lanes 1–3). A significant expression of this mRNA was detected on day 4 of pregnancy (Fig. 2A, lane 4). The level of CTLA-2β mRNAs increased further on days 5–8 and then declined abruptly to an undetectable level by day 10 of pregnancy (Fig. 2A, lanes 5, 6, 8, and 10). The relative level of expression of CTLA-2β was estimated by densitometric scanning, followed by normalization with respect to the control GAPDH mRNA signal. The level of CTLA-2β mRNA on day 4 was about 15-fold higher than that on day 3 of gestation (Fig. 2B). These results indicated that CTLA-2β expression in the pregnant uterus is restricted between days 4 and 10 of gestation, an interval that precisely overlaps with the decidual phase.

We then localized the expression of CTLA-2β by in situ hybridization (Fig. 3). Consistent with the temporal expression pattern described above, the CTLA-2β mRNAs were undetectable between days 1 and 3 of pregnancy (panels A–C). The expression of CTLA-2β was observed on day 5 of pregnancy and this expression was localized exclusively in the stroma surrounding the implanted embryo (panel D). The level of this protease inhibitor in the stromal compartment increased further on days 6 (panel E) and 8 (panel F) and declined sharply on day 9 (panel G) with the cessation of the decidual phase of pregnancy. We also compared the expression of CTLA-2β in the uterine sections of day 5 pregnant animals at implantation and inter implantation sites. Whereas CTLA-2β-specific expression was observed in the uterine sections of day 5 pregnant animals at the implantation sites near the embryo, no CTLA-2β signal was detected at the inter implantation sites (data not shown). Taken together, these results indicated that CTLA-2β is induced in the stromal compartment exclusively at the sites of implantation. The secretion of CTLA-2β from the decidia in the immediate vicinity of the embryo raised the possibility that this factor regulates embryo-uterine interactions during early pregnancy.

Regulation of CTLA-2β Expression: Role of PR-mediated Decidual Signal—The down-regulation of CTLA-2β expression in the pregnant uterus in response to RU486 (Fig. 1) indicated that progesterone acting through PR plays a role in this gene expression. Interestingly, we found that administration of progesterone alone to estrogen-primed ovariectomized mice failed to induce CTLA-2β mRNA in the uterus (data not shown). Since CTLA-2β was expressed in the decidua surrounding the embryo, we investigated whether a progesterone-mediated decidual stimulus is required for CTLA-2β expression.

To induce decidualization, mice (n = 4) were ovariectomized. Two weeks following ovariectomy, mice were administered a hormonal regimen of estrogen and progesterone as described under “Materials and Methods.” Following hormone administration, decidualization was initiated in the right horn by scratching it with a needle while the left horn was undisturbed. As expected, the stimulated horn exhibited marked decidual
response while the unstimulated horn was devoid of any decidual response. The uteri from animals (n = 2) were collected, stimulated or unstimulated horns were separated, and RNA was isolated from these horns for Northern blot analysis using CTLA-2B cDNA as a probe. As shown in Fig. 4, panel A, no signal corresponding to CTLA-2B was observed in the RNA sample obtained from the unstimulated, nondecidualized uterine horn (lane 1). In contrast, a signal of marked intensity was detected in the horn that was decidualized in response to the artificial stimulation (lane 2).

To further analyze the role of PR in CTLA-2B expression in the uterus, we monitored the expression of this gene in the uteri of PR null (n = 2) and wild-type mice (n = 2) of the same genetic background. As shown in Fig. 4, panel B, when these animals were subjected to decidualization using the procedure described above, we failed to observe any signal corresponding to CTLA-2B mRNA in the PR null mice (lane 2). The uterine RNA from wild-type mice, as expected, exhibited the CTLA-2B mRNA signal (Fig. 4, panel B, lane 1). Collectively, these results demonstrated that a PR-induced decidual signal is essential for expression of CTLA-2B in the uterus.

Cathepsin L Is a Target of CTLA-2B in the Pregnant Uterus—

Previous studies indicated that CTLA-2B is a cysteine protease inhibitor (19, 20). A part of the CTLA-2B molecule is highly homologous to the proregions of cysteine proteases, including cathepsins B and L. Consistent with this observation, CTLA-2B binds and inhibits the activity of these proteases in vitro (20). It is, therefore, conceivable that this protease inhibitor might play a role in inhibiting the activity of one or more cysteine proteases secreted by the trophoblast in the decidual uterus.

To determine whether CTLA-2B targets one or more cysteine protease inhibitors in the pregnant uterus, we analyzed its interaction with proteins present in soluble extracts prepared from uteri obtained from mice at days 2 and 7 of pregnancy. The extracts prepared from day 2 pregnant uteri, which did not express CTLA-2B, were unlikely to contain target(s) of this protein and acted as a negative control. In contrast, the extracts from day 7 pregnant uteri, which expressed abundant CTLA-2B mRNA, were likely to contain the target(s) of this protein. To determine whether the target of CTLA-2B in the decidual tissue is of embryonic or maternal origin, we prepared extracts from two kinds of day 7 pregnant uterus: one containing implanted embryos (D7+E) and the other devoid of them (D7−E). A protein affinity strategy employing a recombinant CTLA-2B was used to analyze these uterine extracts. For this purpose, we expressed and purified FLAG epitope-tagged CTLA-2B in baculovirus-infected insect cells (Fig. 5, panel A). The FLAG-CTLA-2B fusion protein was then used to pull down interacting proteins from the day 2 and 7 uterine extracts. The resulting CTLA-2B-associated proteins were run on SDS-PAGE, transferred to a membrane, and probed by immunoblotting using a panel of antibodies against known cysteine proteases, including cathepsins B, D, and L.

We noted that CTLA-2B did not exhibit any interaction with cathepsin B or D in any of the uterine extracts tested (Fig. 5, panel B). We also failed to detect any interaction of this protease inhibitor with cathepsin L in the extracts of day 2 pregnant uteri (lanes 3 and 4, respectively). A cathepsin L-specific signal was, however, clearly visible in the proteins that associated with CTLA-2B in the day 7 uterine extracts containing the embryonic tissue (Fig. 5, panel B, lane 5). Our results, therefore, indicated that cathepsin L is indeed one of the targets of CTLA-2B in the pregnant uterus. Our results also indicated that embryo is the most likely source of cathepsin L.

We also examined the localization of cathepsin L in day 7 pregnant uterus by immunohistochemistry. We observed that cathepsin L was expressed in the day 7 pregnant uteri and its expression was specifically localized to the invasive giant trophoblast cells of the ectoplacental cone (Fig. 5, panel C). Collectively, these results supported our hypothesis that decidual stage-specific expression of CTLA-2B in the pregnant uterus may serve to control embryo implantation by neutralizing the activities of cysteine proteases, such as, cathepsin L, generated by the proliferating trophoblast.

DISCUSSION

In rodents, the progesterone-primed endometrial stroma undergoes extensive remodeling in response to blastocyst implantation, generating a tissue known as the decidua (11-14). The decidua encapsulates the blastocyst and provides an environment within which the trophoblast cells invade, proliferate, and differentiate. The progressive interactions of these two tissues results in the establishment of a highly ordered structure that protects and supports the growth and development of the fetus (11-14). The progesterone-induced signaling molecules that participate in the formation of the decidua tissue and promote its function remain poorly understood.

We identified by DNA microarray analysis a cysteine protease inhibitor, CTLA-2B, which is induced in the uteri of pregnant mice precisely during the decidual phase. The CTLA-2B mRNAs were induced in the mouse endometrium on day 4.5 of pregnancy immediately following embryo attachment to the uterine epithelium, attained a peak during days 5–8, and then declined to undetectable levels after day 10 with the completion of the decidual phase. In situ hybridization analysis revealed that CTLA-2B mRNA expression occurred in an area immedi-
ately surrounding the embryo. It is known that with the onset of implantation, a variety of immune cells, including lymphocytes, natural killer cells, mast cells, and macrophages, converge in the decidua near the implanted embryo (15). At present, we cannot ascertain the nature of cells in the decidua that synthesize CTLA-2β. Nonetheless, the observation that CTLA-2β is secreted in the immediate vicinity of embryo is particularly interesting, and consistent with the proposed role of this factor in regulating embryo-uterine interactions during early pregnancy.

Implantation of the embryo occurs in progressive phases. A role of protease inhibitors in regulating embryo-uterine interactions is consistent with the observation that the final stage of implantation involves invasion of the epithelium by the trophoderm. Trophoblast invasion requires endometrial extracellular matrix proteolysis, as well as cellular migration through the maternal decidua. These processes are precisely regulated and require a balanced interplay between the factors that promote and restrain trophoblast invasion. Progesterone-regulated serine protease inhibitors, which are secreted by endometrial glands during pregnancy, have been known for years. Roberts and co-workers (24, 25) isolated a 14-kDa uterine plasmin-trypsin inhibitor from uterine secretions of pigs during pregnancy (24, 25). Its expression was strongly under the influence of progesterone, and restricted to surface and glandular epithelia. The inhibitor has a well defined Kunitz domain of 64 residues, which shares 67% sequence identity with bovine pancreatic trypsin inhibitor (26). These reports are consistent with the hypothesis that the progesterone-regulated protease inhibitors function by neutralizing the activities of one or more proteases generated by the proliferating trophoblast (27).

In rodents, existing evidence also indicates that factors that promote invasion are primarily trophoblast-derived (28, 29). Consistent with this notion, mouse trophoblast has been shown to synthesize and secrete serine proteases (plasminogen activator), metalloproteases (gelatinase and stromelysin), and the cysteine proteases (cathepsin L) (30–34). The decidual tissue is thought to participate in the control of invasion by secreting the inhibitors of these proteases. Our studies suggest that cathepsin L, secreted by the giant trophoblasts, is one of the targets of CTLA-2β in the pregnant uterus (Fig. 5). Based on these results, we propose that CTLA-2β secreted during the decidual phase regulates trophoblast invasion by neutralizing the activity of trophoblast-derived cathepsin L. An important role of cathepsin L during implantation is suggested by the studies of Babiarz and co-workers (35). They observed that injection of E-64, a synthetic inhibitor of cathepsin L, to pregnant mice affected implantation in a dose-dependent manner. Whereas treatment of mice with a high dose of E-64 resulted in a complete failure of implantation, administration of a low dose of this compound led to stunted embryos and a reduced decidual response. These studies support the view that an optimal action of cathepsin L within the decidua is critical for the decidualization and implantation processes.

CTLA-2β was initially isolated in the process of identification of gene products important for the function of cytotoxic T lymphocytes (19, 36). It is, therefore, conceivable that uterine CTLA-2β may also play a role in the regulation of T cell action during early pregnancy. At the time of embryo implantation, the maternal response to the invading semi-allograft embryo

![Fig. 4. Expression of CTLA-2β mRNA in mouse uterus during artificially induced decidualization. A, CD-1 (n = 4) mice were ovariectomized and subjected to artificial decidualization as described under “Materials and Methods.” Northern blot analysis of CTLA-2β using RNA from unstimulated (lane 1) and stimulated (lane 2) uterine horn. B, 129 (n = 2) and PRKO (n = 2) mice were ovariectomized and subjected to artificial decidualization as described under “Materials and Methods.” Northern blot analysis of CTLA-2β using RNA from wild-type (lane 1) and PRKO (lane 2) uterine horn followed decidual stimulation.](http://www.jbc.org/content/181/15/10361/F4)

using RNA from unstimulated (lane 1) and stimulated (lane 2) uterine horn. B, 129 (n = 2) and PRKO (n = 2) mice were ovariectomized and subjected to artificial decidualization as described under “Materials and Methods.” Northern blot analysis of CTLA-2β using RNA from wild-type (lane 1) and PRKO (lane 2) uterine horn followed decidual stimulation.
has the characteristics of an acute inflammation (37, 38). However, once implanted, the embryo suppresses this response and prevents rejection. Simultaneously, the immune system of the mother prevents a graft versus host reaction that might derive from the fetal immune system (37, 38). Consistent with this notion, recent studies have shown that agents, which facilitate killing of activated T cells, promote implantation and maintenance of early pregnancy (39, 40). It is likely that maternal immune response to the invading embryo is kept in check via regulation of T cell repertoire.

Interestingly, a number of recent studies indicated that cathepsin L plays an important role in T cell selection (41, 42). A critical step in major histocompatibility complex class II-restricted antigen presentation is degradation of the invariant chain (Ii). Cathepsin L was found to be necessary for Ii degradation, and consequently, cathepsin L null mice exhibited a reduced number of CD4+ T cells (~60–80% reduction) in the thymus and periphery (41, 42). Consistent with this observation, a CD4+ T cell-deficient mouse was found to harbor a deletion mutation of the cathepsin L gene (43). Interestingly, our findings in this article raise the possibility that CTLA-2β synthesized in the decidua surrounding the implanting embryo may inhibit the activity of cathepsin L produced by the embryo, leading to deregulation of major histocompatibility complex class II processing. This in turn, may lead to a reduction in the number of certain subtypes of T cells, thus facilitating the modulation of maternal immune response to the invading embryo. Future studies will determine whether the principal function of CTLA-2β in the pregnant uterus is to interact with cathepsin L to control trophoblast invasion and/or modulate T cell function to control maternal immune response at the time of implantation.

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