Presence and regulation of messenger ribonucleic acids encoding components of the class II major histocompatibility complex-associated antigen processing pathway in the bovine corpus luteum

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Abstract

Luteal cells express class II major histocompatibility complex (MHC) molecules and can stimulate T lymphocyte proliferation in vitro. However, it is unknown whether luteal cells express the intracellular components necessary to process the peptides presented by class II MHC molecules. The objective of the present study was to examine the expression and regulation of three major class II-associated antigen processing components – class II MHC-associated invariant chain (II), DMα and DMβ – in luteal tissue. Corpora lutea were collected early in the estrous cycle, during midcycle and late in the estrous cycle, and at various times following administration of a luteolytic dose of prostaglandin F₂α (PGF₂α) to the cow. Northern analysis revealed the presence of mRNA encoding each of the class II MHC-associated antigen processing proteins in luteal tissue. II mRNA concentrations did not change during the estrous cycle, whereas DMα and DMβ mRNA concentrations were highest in midcycle luteal tissue compared with either early or late luteal tissue. Tumor necrosis factor-α (TNF-α) reduced DMα mRNA concentrations in cultured luteal cells in the presence of LH or PGF₂α. DMα and DMβ mRNA were also present in highly enriched cultures of luteal endothelial (CLENDO) cells, and DMα mRNA concentrations were greater in CLENDO cultures compared with mixed luteal cell cultures. Expression of invariant chain, DMα and DMβ genes indicates that cells within the corpus luteum express the minimal requirements to act as functional antigen-presenting cells, and the observation that CLENDO cells are a source of DMα and DMβ mRNA indicates that non-immune cells within the corpus luteum may function as antigen-presenting cells.

Introduction

providing a means of activation of T lymphocytes present in luteal tissue.

Class II MHC molecules, which are typically expressed on antigen-presenting cells of the immune system (i.e. macrophages, dendritic cells and B lymphocytes), present small peptides processed intracellularly to T lymphocytes (Sant 1994). When cells expressing class II MHC molecules (antigen-presenting cells) interact with T cells, the peptides bound to the MHC molecules determine whether the antigen-presenting cell will activate T cells. The immune system is tolerant of normally expressed self-peptides, but T lymphocytes can be activated by cells expressing peptides derived from a foreign source (i.e. viral or bacterial peptides) as well as self-peptides that are presented by MHC molecules under abnormal conditions (i.e. tumor antigens). As class II MHC molecules are synthesized, they immediately bind to a chaperone protein called invariant chain (II), a portion of which occupies the peptide-binding groove of the MHC molecule (Roche & Cresswell 1990, Avva & Cresswell 1994). Invariant chain serves a dual function, directing intracellular transport of class II MHC molecules (Bakke & Dobberstein 1990, Lotteau et al. 1990) and ensuring that binding of ‘inappropriate’ peptides derived from a foreign source (Roche & Cresswell 1990). Class II MHC–II complexes are transported into specialized intracellular compartments in which proteases degrade the II into a peptide called class II-associated invariant chain peptide (CLIP) (Maric et al. 1994, Riese et al. 1996). Prior to binding of antigenic peptides by class II MHC molecules, the CLIP fragment bound to the peptide binding groove must be removed. Removal of CLIP is carried out by a class II MHC-like heterodimeric protein referred to as DM (Fling et al. 1994, Morris et al. 1994). Following CLIP removal, DM stabilizes class II MHC molecules to allow efficient binding of antigenic peptides (Denzin et al. 1996).

In addition to the primary roles for II and DM described above, these intracellular proteins have been implicated in a secondary role as ‘editors’ of the repertoire of peptides presented to T cells in the context of class II MHC, since they control in part the type of peptides that bind to class II MHC molecules and that are presented to T lymphocytes (Castellino et al. 1997, Vogt et al. 1999). Antigen-presenting cells taken from Ii or DM knockout mice express the components of the class II MHC antigen-processing pathway.

In expression of class II MHC genes and associated antigen-processing genes may also be involved in the enhancement of luteal cell-stimulated T cell proliferation, and that processing of antigens by luteal cells may be altered near the time of luteal regression. Alteration of antigen processing within luteal cells could result in binding of a different repertoire of peptides to class II MHC molecules, allowing luteal cells to stimulate T cell activation during luteal regression. The hypothesis tested in the present study is that expression of either II or DM changes near the end of the estrous cycle. Due to observations in the initial portion of the present study, we wished to determine whether steady-state concentrations of DMea and DMβ mRNA are regulated by luteinizing hormone (LH) and PGF2α, the classic hormonal regulators of luteal function, or by TNF-α, which is suspected to play a significant role in regulation of luteal function. We have also examined highly enriched cultures of luteal endothelial (CLENDO) cells for the presence of DMea and DMβ mRNA, to determine whether endothelial cells of the CL express the components of the class II MHC antigen-processing pathway.

**Materials and Methods**

**Reagents**

Powdered Ham’s F-12 culture medium, gentamicin, fetal bovine serum, *Escherichia coli* DH5α chemically competent cells, and TRIzol Reagent were all purchased from Life Technologies (Grand Island, NY, USA). Recombinant murine TNF-α was purchased from Life Technologies and R&D Systems (Minneapolis, MN, USA). The National Hormone and Pituitary Program (Baltimore, MD, USA) provided bovine LH (NIAMMD-bLH-4). Insulin-transferrin-selenium (ITS) premix was obtained from BD Biosciences (Bedford, MA, USA). Bovine serum albumin (fraction V), PGF2α, HEPES buffer and Bouin’s fixative were purchased from Sigma. Preix fixative was purchased from Anatrace Ltd (Battle Creek, MI, USA). Type I collagenase was acquired from Worthington Biochemical Corp. (Freehold, NJ, USA). SDS and MOPS buffer were acquired from Amresco (Solon, OH, USA). Digoxigenin-labeled rNTP mix, alkaline-phosphatase-conjugated anti-digoxigenin Fab fragments, CDP-Star chemiluminescent substrate, blocking
Expression of class II MHC antigen processing genes in the bovine corpus luteum

Fifteen micrograms of total cellular RNA were electrophoretically separated on 1.5% agarose denaturing gels containing 20 mM MOPS buffer, 5 mM sodium acetate, 1 mM EDTA and 0.66 M formaldehyde. Following electrophoresis, gels were stained for 15 min with 1 μg/ml ethidium bromide in DEPC-treated water, destained for 1 h in DEPC-treated water and photographed. RNA was transferred to Hybond-N+ membranes, and membranes were baked at 80°C for 2 h to crosslink RNA to the membranes. Gels were restained with ethidium bromide following transfer and examined under u.v. light to confirm complete transfer of RNA.

Digoxigenin-labeled riboprobes were synthesized using plasmids containing full-length Bovine Lymphocyte Antigen (BoLA)-DMα, BoLA-DMβ or bovine class II MHC-associated li cDNA sequences. Plasmids containing these sequences have been described previously (Niimi et al. 1995, 1996) and were provided by Dr Yoko Aida (The Institute of Physical and Chemical Research, Tsukuba, Japan). Digoxigenin-labeled riboprobes were generated using linearized plasmids according to the instructions in Roche Molecular Biochemicals’ DIG Nonradioactive Nucleic Acid Labeling and Detection System manual. Membranes were prehybridized at 68°C for 1 h with hybridization core buffer (250 mM sodium phosphate, 1 mM EDTA, 5% (w/v) SDS containing 0.5% (w/v) Roche DIG Nucleic Acid Detection System Blocking Reagent), and hybridization was carried out for 16 h at 68°C. Following hybridization, membranes were washed and detection was performed using alkaline phosphatase-conjugated anti-digoxigenin Fab fragments in concert with the chemiluminescent CDP-Star substrate, according to the specifications in Roche Molecular Biochemical’s DIG Nonradioactive Nucleic Acid Labeling and Detection System manual. Membranes were exposed to Biomax ML film (Eastman Kodak Co., Rochester, NY, USA) to detect chemiluminescence.

Quantification of steady-state concentrations of each message was performed densitometrically using a PDI 420oe scanning densitometer. The densitometric value (in arbitrary densitometric units) of the band of interest in each sample was standardized to the densitometric values of the corresponding 18S rRNA.

RNA isolation and Northern analysis

Frozen tissue collected for the first experiment was homogenized in TRIzol reagent using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY, USA). In the second experiment, RNA from cultured cells was isolated by adding TRIzol reagent directly to culture flasks. Following tissue homogenization or cell lysis, total cellular RNA was isolated according to procedures outlined by the manufacturer. The final RNA precipitate was resuspended in double-distilled water containing 0.1% diethyl pyrocarbonate (DEPC) and RNA concentration was determined spectrophotometrically.

Animals and tissue collection

Multiparous, lactating dairy cows between 3 and 6 years of age were used in the present studies. Animals were housed indoors and had complete freedom of movement; they were given regular access to a total mixed ration and were allowed to feed ad libitum. Corpora lutea were removed transvaginally from regularly cycling dairy cows. Blood samples were collected from all animals prior to CL removal to determine circulating concentrations of progesterone. Corpora lutea were cut into four equal pieces, three of which were immediately snap frozen in liquid nitrogen, transported to the laboratory and stored at −80°C until RNA extraction was performed. In the first experiment, CL were removed early in the estrous cycle (day 5, n = 6), during midcycle (days 9–12, n = 6) or late in the estrous cycle (day 18, n = 6), or at 0.5 (n = 5), 1 (n = 5), 4 (n = 5), 12 (n = 4) or 24 h (n = 5) following i.m. administration of 25 mg PGF2α (Lutalyse; Upjohn Co., Kalamazoo, MI, USA). In the second experiment, CL were removed during midcycle, immediately placed in ice-cold Ham’s F-12 culture medium on ice and transported to the laboratory for dissociation. Handling of animals and surgical procedures were carried out in accordance with protocols approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Luteal cell dissociation and culture

Dissociation of luteal cells was carried out according to procedures described previously (Pate & Condon 1982). Cell culture was performed in a humidified atmosphere of 5% CO2 in air at 37°C. Dispersed luteal cells (4 x 10⁶ cells/flask) were cultured in Ham’s F-12 medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), gentamicin (20 μg/ml) and LH (1 ng/ml) in serum-coated 25 cm² flasks, in a total of 4 ml of culture medium. Cells were allowed to adhere overnight, medium was replaced and cultures were treated with LH (10 ng/ml), TNF-α (50 ng/ml) or PGF2α (10 ng/ml) in a...
3 x 3 factorial arrangement. Treatment concentrations used in this study have been shown previously to affect function, viability and gene expression in cultured bovine luteal cells (Pate & Condon 1984, Townson & Pate 1994, Petroff et al. 2001, Cannon & Pate 2003). Medium and treatments were replaced after 24 h. Total RNA was extracted from cultured cells after 48 h of culture. The experiment was replicated a total of four times using CL from different animals.

Isolation and culture of purified luteal endothelial cells
Purified endothelial cells from bovine CL (CLENDO cells) collected during early pregnancy were purchased from Cambrex BioScience (BioWittaker, Walkersville, MD, USA) as described previously (Cavicchio et al. 2002, Pru et al. 2003, Liptak et al. 2005). In the present study, endothelial cells from frozen aliquots (passages 3–5; 5000 cells/cm²) were cultured in EGM-2MV media, as recommended by the supplier with 3% fetal bovine serum (Cambrex Bioscience) in 60 mm dishes. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Culture medium was replaced every 48 h until 80–90% confluent. On the day of harvest, cells were equilibrated for 2–3 h in serum-free EBM-2 medium. CLENDO cells were collected in RLT lysis buffer and RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications.

Quantitative reverse transcription-PCR (RT-qPCR)
Primers used to amplify DMα and DMβ cDNAs were designed based on the published sequences for DMα and DMβ (Niimi et al. 1995; GenBank accession numbers D76416 and D76417 respectively). Primer sequences were 5'-GCTCTCTGAGTCTACGAGA-3' and 5'-GCACAGCACATTCTCCAGAG-3' for DMα forward and reverse primers respectively, and 5'-CACCCTGTTGTGGAGCAGATGA-3' and 5'-AAGCCTACACAGACGACAGAG-3' for DMβ forward and reverse primers respectively. Amplification using DMα primers resulted in a single 526 bp DMα cDNA fragment, whereas amplification using DMβ primers resulted in a single 597 bp DMβ cDNA fragment. Primer sequences used for amplification of steroidogenic acute regulatory protein (StAR) and CD31 (an endothelial cell adhesion molecule) mRNA were 5'-GTGATGACCGTG-3' and 5'-GCTCTCTGAGTCTACGAGA-3' for forward and reverse primers respectively; 5'-GGCAAGCTGCTGAGGAG-3' and 5'-CCTCTCTCAAGGACCAA-3' for StAR forward and reverse primers respectively. Amplification using the CD31 primers resulted in a 229 bp CD31 cDNA product and amplification using StAR primers yielded a 311 bp StAR cDNA product. The identity of all PCR products was confirmed by DNA sequence analysis. An 854 bp glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA fragment was amplified in parallel reactions, using ovine specific primers described previously (Tsai et al. 1996), and concentrations of DMα, DMβ, CD31 and StAR in each sample were standardized to the corresponding G3PDH concentrations.

Steady-state concentrations of mRNA encoding StAR, CD31, DMα and DMβ were determined in total RNA extracted from cultures of mixed luteal cells and luteal endothelial cells, using an MJ Research Opticon 2 real-time PCR thermal cycler. Prior to PCR, reverse transcription using random hexamer primers was performed on 2 μg of total RNA extracted from cultures. PCR was then performed, using 200 ng of reverse transcribed cDNA, using the DyNaMo HS SYBR Green qPCR kit according to the manufacturer's instructions. Thermal cycling was carried out using the following conditions: denaturation, 94°C for 30 s; annealing, 56°C for 30 s; extension, 72°C for 60 s, for a total of 32 cycles. Melting curve analysis was performed after the end of the last cycle and, in conjunction with gel electrophoresis of amplified products, was used to verify the amplification of a single product in each sample. Fluorescence values in each tube were measured at the end of each cycle using single acquisition mode. Fluorescence values of the product of interest in each sample were standardized to the corresponding G3PDH fluorescence values, and these standardized values were then used to calculate the mean steady-state amounts of each message. Since no standard curve was run for the messages of interest, values were expressed in arbitrary units of fluorescence for the purposes of analysis. Steady-state concentrations of DMα and DMβ mRNA were determined in total RNA extracted from luteal cells treated with LH, PGF₂α, and TNF-α using a Roche LightCycler real-time PCR thermal cycler. Prior to quantitative PCR, DMα, DMβ or G3PDH reverse primer was used to reverse transcribe 200 ng of total cellular RNA extracted from cultured cells. Quantitative PCR was then performed on 40 ng of reverse transcribed RNA, using the Roche LightCycler-FastStart DNA Master SYBR Green I kit according to the manufacturer's instructions. As a standard curve, DMα, DMβ and G3PDH cDNAs were amplified from lymph node total RNA, electrophoretically separated on a 1.2% agarose gel and eluted. The concentration of purified cDNA products was determined spectrophotometrically, and serial dilutions were prepared and used as an external standard curve in the quantitative PCR assays. Thermal cycling was carried out using the following conditions: denaturation, 94°C for 30 s; annealing, 58°C for 30 s; extension, 72°C for 60 s, 40 cycles total. The ramping speed for transition from denaturation to annealing steps and annealing to extension steps was set manually at 1°/s, while the ramping speed from extension to denaturation steps was set at 20°/s to minimize total running time. Melting curve analysis was performed after the end of the last cycle in order to verify the amplification of a single
product in each sample. There was no evidence of primer dimers or bands other than the desired amplicons in the reactions as determined by melting curve analysis, and this was confirmed by gel electrophoresis of amplified products. The lack of undesired products allowed fluorescence measurements to be taken at the same temperature (72°C) at which extension was performed. Fluorescence values in each tube were measured at the end of each cycle using single acquisition mode. Samples were run in duplicate and fluorescence values for duplicate samples were averaged. The baseline fluorescence reading and noise band cut-off were set manually for samples and standards, in order to eliminate background fluorescence values from calculation of the slope and y-intercept of the standard curve. A standard curve was generated using average fluorescence values of duplicate standards. Average fluorescence values of samples were then used to calculate the concentration of target cDNA in each amplification reaction, using the Fit Points methods of the accompanying LightCycler data analysis software.

**Statistical analysis**

Normalized densitometric values collected from Northern analyses in the first experiment were subjected to two-way analysis of variance to determine whether differences in mean densitometric values of DMα, DMβ, or li mRNA existed between stages of the estrous cycle. In the instance of a significant F value, the Student–Newman–Kuels (S–N–K) test was used to determine differences between specific means. Relative differences in steady-state concentrations of CD31, StAR, DMα and DMβ mRNA between cultures of mixed luteal cells or luteal endothelial cells were determined using Student's t test. In the third experiment, steady-state concentrations of DMα or DMβ mRNA in treated luteal cell cultures were subjected to one-way ANOVA to determine if differences existed between treatment means. In the incidence of a significant F value, the S–N–K test was used to determine differences between specific treatment means. Differences were considered to be significant at P < 0.05. A P value between 0.05 and 0.08 was considered to show a tendency towards a significant difference. All statistical procedures were performed using the SigmaStat statistical analysis software package (Jandel Corporation, San Rafael, CA, USA).

**Results**

The results of the Northern analyses to detect and quantify li, DMα and DMβ mRNA in luteal tissue are displayed in Figs 1 to 3. Figure 1A demonstrates the presence of an li transcript of approximately 1.3 Kb in all luteal tissue samples. Muscle tissue, which served as a source of negative control RNA, was devoid of any corresponding band (not shown). There was a large amount of variability among animals in the steady-state concentration of li mRNA following in vivo administration of PGF2α. This is responsible for the apparent discrepancy between the density of bands in the Northern blot shown in Fig. 1A and the graph shown in Fig. 1B, which represents the mean of data from four or five animals at each time after PGF2α administration. There were no differences in concentrations of li mRNA between CL collected at any stage of the estrous cycle; however there was a greater than two-fold increase in steady-state concentrations of li mRNA in CL collected 24 h after PGF2α administration (P < 0.05, Fig. 1B). Figure 2A shows a Northern blot of DMα mRNA demonstrating the presence of a single DMα transcript in luteal tissue. Muscle tissue RNA was devoid of any corresponding band (not shown). Similar to the results of li Northern analysis, a large amount of variability among animals was also found in DMα mRNA concentrations following PGF2α administration, which is responsible for the apparent difference between the density of bands in the Northern blot shown in Fig. 2A and the graph shown in Fig. 2B, which represents the mean of data from four or five animals at each time after PGF2α administration. Such variability was not observed among animals in CL collected during the estrous cycle. Concentrations of DMα mRNA tended (P > 0.08) to be greater in CL collected during midcycle compared with CL collected early (day 5) or late (day 18) in the estrous cycle (Fig. 2B). There were no differences (P > 0.10) in concentrations of

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Northern analysis of li mRNA in bovine luteal tissue. (A) Northern blot showing li mRNA in a single CL collected from each of the following times of the estrous cycle: early (day 5, n = 6); during midcycle (days 10–12, n = 6); or late (day 18, n = 6); and at 0 (n = 5), 0.5 (n = 5), 1 (n = 5), 4 (n = 5), 12 (n = 4) or 24 h (n = 5) following administration of 25 mg PGF2α to the cow. 18S rRNA corresponding to each sample is also shown. (B) Steady-state concentration of li mRNA in luteal tissues collected at each time point (mean ± S.E.M., n values as stated above). Bars represent densitometric values of li mRNA standardized to 18S rRNA. Values with different letters are significantly different (P < 0.05).
DMα mRNA in CL collected following administration of a luteolytic dose of PGF$_{2\alpha}$, possibly due to the large degree of animal-to-animal variation mentioned earlier. Messenger RNA encoding DMβ was also detected in luteal tissue by Northern analysis, as demonstrated by the presence of a single DMβ transcript in all luteal tissue samples (Fig. 3A). Muscle tissue RNA was also devoid of any band corresponding to DMβ (not shown). Concentrations of DMβ mRNA were greater ($P < 0.05$) in midcycle CL as compared with CL collected early or late in the estrous cycle (Fig. 3B), similar to DMα. There were no differences in concentrations of DMβ mRNA following PGF$_{2\alpha}$ administration.

Steady-state concentrations of DMα and DMβ mRNA in CLENDO cells and mixed luteal cells were compared in order to determine whether endothelial cells derived from the bovine corpus luteum expressed DMα and DMβ. Purity and composition of the CLENDO cell cultures and mixed luteal cell cultures was assessed using quantitative RT-PCR to determine steady-state concentrations of mRNA encoding CD31 and StAR protein. CD31 mRNA was present both in mixed luteal cell and CLENDO cultures, with much greater steady-state concentrations present in CLENDO cultures. StAR mRNA, while abundant in mixed luteal cell cultures, was undetectable in some CLENDO cultures, and was present in concentrations near the detection limit of the PCR assay in others (data not shown). Messenger RNA encoding DMα and DMβ was detectable in cultures of mixed luteal cells as well as CLENDO cell cultures. Steady-state concentrations of DMα, but not DMβ, mRNA were greater in CLENDO cultures (Fig. 4).

The effects of LH, PGF$_{2\alpha}$ and TNF-α on the concentrations of DMα mRNA in cultured bovine luteal cells are displayed in Fig. 5. Concentrations of DMα were less in TNF-α-treated cultures compared with controls ($P < 0.05$). Similarly, in the presence of LH or PGF$_{2\alpha}$, TNFα caused a reduction in DMα mRNA concentrations. Treatment with TNF-α in the presence of both LH and PGF$_{2\alpha}$ also caused slight, but non-significant, reductions in DMα mRNA. LH, PGF$_{2\alpha}$ and TNF-α were all without effect on concentrations of BoLA-DMβ mRNA in cultured luteal cells (Fig. 5).

**Discussion**

The present study is the first to demonstrate the presence of mRNA encoding li, DMα and DMβ in the CL of any species. These results are significant when considering the increase in the ability of luteal cells to stimulate T cell proliferation in response to PGF$_{2\alpha}$. Although we have previously demonstrated that reduction in progesterone synthesis is at least partly responsible for the increase in the ability of luteal cells to stimulate T lymphocyte proliferation following PGF$_{2\alpha}$ administration (Cannon et al. 2003), alteration in intracellular antigen processing could
also contribute to the enhanced ability of luteal cells from regressing CL to stimulate T cell proliferation. Alterations in DMα and DMβ expression near the end of the estrous cycle could catalyze a change in luteal cells that facilitates the process of luteal regression.

Steady-state concentrations of li mRNA were not different at any stage of the estrous cycle. The reason for this is not clear. Class II MHC expression on bovine luteal cells increases throughout the estrous cycle and following PGF2α administration (Benyo et al. 1991), and since li is required for transport of class II MHC molecules to the cell surface, expression of li would be expected to parallel class II MHC expression. Given the observed lack of change in li mRNA during the estrous cycle or following administration of PGF2α, it appears that this gene is constitutively expressed in luteal tissue. It is possible that constitutive expression of li is sufficient to allow transport of class II MHC molecules to the cell surface. It must be noted, however, that the present study measured mRNA whereas the previous study measured cell surface proteins (Benyo et al. 1991). In that study, expression of class II MHC molecules by luteal cells was not measured beyond 12 h after a luteolytic dose of PGF 2α. It is possible that class II MHC expression may increase dramatically by 24 h after PGF2α, similar to the increase in li mRNA concentrations observed in the present study.

Concentrations of DMα and DMβ mRNA were greatest in the CL during midcycle, and declined near the time of luteal regression. Since previous studies have shown that li and DM are coordinately regulated by the class II trans-activating factor CIITA (Steimle et al. 1994, Chang & Flavell 1995), this result was unexpected in light of the observed lack of change in li mRNA concentration throughout the estrous cycle. The number of bovine luteal cells expressing class II MHC molecules, as well as the degree of class II MHC expression, both increase near the time of luteal regression (Benyo et al. 1991). The decline in DMα and DMβ mRNA concentrations in luteal tissue concurrent with increases in class II MHC expression may result in presentation of a repertoire of peptides to T cells during luteal regression that is different from that presented during midcycle. Administration of a luteolytic dose of PGF2α to the cow did not induce changes in the steady-state concentrations of DMα or DMβ mRNA. The reason for the lack of effect of PGF2α on DMα and DMβ expression is unknown, but it is evident that the control of expression of these genes in the CL is not regulated by PGF2α. Treatment of cultured luteal cells with TNF-α reduced concentrations of DMα, and elevation of TNF-α during luteal regression has been demonstrated (Ji et al. 1991, Shaw & Britt 1995). Elevation of TNF-α in the CL was not observed until after the decline in circulating and tissue concentrations of progesterone, but intra-luteal concentrations of TNF-α may increase prior to the decline in circulating concentrations of progesterone, which would explain the decline in DMα mRNA observed in late cycle CL. Steady-state concentrations of DMβ mRNA were not
affected by LH, TNF-α or PGF₂α, indicating that multiple factors control the expression of DM in the CL.

The present study demonstrates the presence of Ii, DMα and DMβ mRNA in luteal tissue in amounts detectable using the relatively non-sensitive method of Northern analysis. That these messages are detectable by Northern analysis is suggestive of a source of mRNA other than infiltrating macrophages and B lymphocytes, which are relatively few in number compared with the total number of cells in the bovine CL. DMα and DMβ mRNA were also found in CLENDO cultures in the present study, which provides support for the hypothesis that a cell type in addition to macrophages in the CL expresses these components of the antigen-processing pathway. It is worth noting that the CLENDO cultures used in the present study are composed of very highly purified populations of luteal endothelial cells, since the concentration of CD31 mRNA in CLENDO cells was approximately ten-fold greater than in mixed luteal cell cultures, whereas StAR mRNA is almost undetectable. Conversely, the mixed luteal cell cultures contain largely steroidogenic cells, but are likely to also contain small numbers of endothelial cells; large amounts of StAR mRNA were present in the mixed luteal cell cultures, whereas concentrations of CD31 mRNA were much less than those in CLENDO cultures. Steady-state concentrations of DMα were greater in CLENDO cell cultures compared with mixed luteal cell cultures, and although it was not significant, concentrations of DMβ mRNA also appeared to be greater in CLENDO cell cultures compared with mixed luteal cell cultures. However, DMα and DMβ mRNAs were also present in mixed luteal cell cultures in relatively high abundance. From the analysis of CD31 concentrations in the CLENDO and mixed luteal cell cultures, we have concluded that endothelial cells make up a very small proportion of the total cells in mixed luteal cell cultures. Therefore, it seems unlikely that endothelial cells account for the relatively large amount of DMα and DMβ mRNA present in mixed luteal cell cultures. We have not assessed the presence of macrophages in these cultures, but while it is unlikely that macrophages are present in the CLENDO cultures, due to the purification procedures used to derive these cultures, it is possible that significant numbers of macrophages are present in mixed luteal cell cultures. This would account for the presence of the relatively large amount of DMα and DMβ mRNA found in mixed luteal cell cultures. It is unfortunate that it was not possible to obtain a pure population of steroidogenic luteal cells for these studies, and it is not possible to conclude from this experiment whether or not steroidogenic cells express DMα and DMβ. However, these results clearly demonstrate that luteal endothelial cells express DMα and DMβ.

In conclusion, the present study demonstrates the expression of Ii, DMα and DMβ genes in luteal tissue. These genes encode intracellular antigen-processing proteins required for processing and presentation of antigenic peptides to T lymphocytes. The changes in DMα and DMβ expression observed near the end of the estrous cycle could result in presentation of an altered repertoire of self-peptides in the context of class II MHC molecules. This alteration of antigen presentation may have significant implications for regulation of luteal function via interaction of resident T lymphocytes with cells of the CL. However, since expression of the antigen-processing components examined in this study is greatest during mid-cycle, it is possible that presentation of antigen by class II MHC is necessary in the fully functional CL to maintain a state of immune tolerance to the tissue. Since immune tolerance would no longer be essential at the end of the estrous cycle, the decline in DMα and DMβ expression may serve to facilitate activation of immune cells that may participate in the process of regression. Future studies are needed to determine the role(s) played by the immune system in regulation of luteal function.

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