Immunohistochemical localization of the LH/HCG receptor in human ovary: HCG enhances cell surface expression of LH/HCG receptor on luteinizing granulosa cells in vitro

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We examined the immunohistochemical localization of luteinizing hormone (LH)/human chorionic gonadotrophin (HCG) receptor (LH-R) in the human ovary using the anti-human LH-R monoclonal antibody, 3B5. In the antral follicles, LH-R was detected on theca interna cells. In pre-ovulatory follicles, granulosa cells also expressed LH-R. During corpus luteum formation, granulosa cells seemed to increase the expression of LH-R, and in corpus luteum of mid-luteal phase, large luteal cells expressed LH-R more intensely than small luteal cells. In the regressing corpus luteum, LH-R was almost undetectable on both luteal cells, whereas in the corpus luteum of early pregnancy, LH-R continued to be expressed on large luteal cells. The granulosa cells obtained from the patients undergoing in-vitro fertilization therapy were cultured for 3 days in serum-free medium, without or with HCG (10 IU/ml) and tumour necrosis factor (TNF)α (10 ng/ml). Flow cytometry showed that the expression of LH-R on the cell surface of luteinizing granulosa cells was enhanced by HCG, but was unaffected by TNFα. These results suggest that the main target cells for LH/HCG change from theca interna cells/small luteal cells to granulosa cells/large luteal cells during ovulation, corpus luteum formation, and differentiation into the corpus luteum of pregnancy, probably under the influence of LH/HCG.

Key words: luteinizing hormone/ovarian cells/receptor

Introduction

In human follicles, it is thought that the thecal layer is the major cellular source of follicular androgen and that luteinizing hormone (LH) stimulates thecal androgen production (Tsang et al., 1979; Nahum et al., 1995). In addition, LH is thought to stimulate the production of granulosa cells in pre-ovulatory follicles (McNatty et al., 1979). LH and human chorionic gonadotrophin (HCG) are also considered to be important luteotrophic factors for the formation of the mature corpus luteum and for the promotion to the corpus luteum of pregnancy, respectively.

To elucidate the physiological role of LH/HCG on the follicles or differentiation of follicles and corpus luteum, many investigators have reported the expression profiles of LH/HCG receptor (LH-R) in the ovary. Although the reported expression profiles of LH-R in the follicles are almost similar between species, there is an apparent discrepancy in those of LH-R in corpus luteum between species. In rats, immunoreactive LH-R is demonstrated to be localized on luteal cells in corpus luteum of the cycle and pseudo-pregnancy using an anti-rat LH-R monoclonal antibody (Bukovsky et al., 1993). In bovine corpus luteum, both large luteal cells and small luteal cells had ¹²⁵I-HCG-binding sites with the highest level of binding during the mid-luteal phase (Chegini et al., 1991). In contrast, in porcine ovaries immunoreactive LH-R is expressed on the most external luteal cells in the corpus luteum, but not on internal luteal cells, probably corresponding to small luteal cells and large luteal cells respectively (Meduri et al., 1992). This discrepancy may be due to the differences in the physiological roles of LH and HCG on corpus luteum function and differentiation among various species.

In the human corpus luteum, it is reported that LH binding sites on granulosa cells/large luteal cells increase from the early luteal phase until they reach the highest level in the mid-luteal phase, then decrease toward the late luteal phase, and are undetectable in early pregnancy by means of autoradiography (Shima et al., 1987). In contrast, mRNA studies revealed that mRNA of LH-R is expressed in early pregnancy as high as in the early luteal phase (Nishimori et al., 1995), indicating that the expression of LH-R as a protein product in human corpus luteum, especially during pregnancy, remains unclear. Immunohistochemical studies have merits in that a protein product is detected by a specific antibody and in that the distribution of the protein seems to be better understood than by autoradiography or mRNA study. Bukovsky et al. (1995) examined the expression of LH-R in the human menstrual corpus luteum by immunohistochemistry, but they did not describe the difference in LH-R expression between large and small luteal cells, nor did they examine LH-R expression in follicles or in the corpus luteum of pregnancy in detail. Thus, target cells of LH/HCG in human ovary are not fully clarified. In this study, we examined the immunohistochemical...
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Figure 1. The expression of LH-R in small follicles detected by indirect immunofluorescence staining. A-C: a preantral follicle containing three or four layers of granulosa cells. D-F: a small antral follicle <1 mm in diameter. A and D: haematoxylin and eosin staining. B and E: LH-R expression stained by 3B5 mAb. C and F: negative controls stained by anti-TNP mAb. B: LH-R was moderately expressed on some theca interna cells. E: LH-R was moderately expressed on theca interna cells. GC: granulosa cells. TI: theca interna cells. Oc: oocyte. Original magnification, A-C: x240, D-F: x120.

Localization of LH-R in human follicles and the corpus luteum of the menstrual cycle and of early pregnancy using an anti-rat LH-R mAb (3B5) that cross-reacts with human LH-R (Bukovsky et al., 1993). To evaluate the role of LH/HCG on the corpus luteum formation, the effects of HCG and tumour necrosis factor (TNF)α on expression of LH-R on the cell surface of cultured human luteinizing granulosa cells were also examined by flow cytometry.

Materials and methods

Antibodies

The murine anti-human and rat LH-R mAb 3B5 (Bukovsky et al., 1993), and the negative control, murine anti-trinitrophenyl (TNP) mAb (Tsujimura et al., 1990) were diluted in RPMI 1640 (ICN Biochemicals, Costa Mesa, CA, USA) with 10% fetal calf serum (FCS) (Flow Laboratories, McLean, VA, USA) and 0.1% NaN₃.

Human ovaries

Human ovarian follicles and corpora lutea of the menstrual cycle were obtained from 45 patients (14–42 years) with regular menstrual cycles, undergoing surgery for benign disease. Corpora lutea of pregnancy (6–15 weeks’ gestation) were obtained from seven pregnant patients who underwent hysterectomy for the treatment of cervical carcinoma in situ or uterine myoma. In these pregnant patients, intrauterine fetal growth was normal upon ultrasonographic examination. Informed consent was obtained from each patient.

Growing and atretic follicles were morphologically evaluated by staining cryosections with haematoxylin and eosin. Follicles obtained in the follicular phase, of which the granulosa cells had regularly shaped nuclei, cytoplasm, stratified layers and mitotic figures, were classified as growing follicles. Follicles that were irregularly shaped, showed blood cell invasion and lacked mitotic figures were classified as atretic (Ryan, 1981). For corpus luteum, the post-ovulatory date was re-evaluated according to histological dating (corpus luteum day), using haematoxylin and eosin-stained tissue sections of 10% formalin-fixed and paraffin-embedded samples.
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Figure 2. The expression of LH-R in growing and pre-ovulatory follicles detected by indirect immunofluorescence staining. A-C: a growing follicle 4 mm in diameter. D-F: a pre-ovulatory follicle 20 mm in diameter. A and D: haematoxylin and eosin staining. B and E: LH-R expression stained by 3B5 mAb. C and F: negative controls stained by anti-TNP mAb. B: LH-R was moderately expressed on theca interna cells, but not on granulosa cells. E: LH-R was intensely expressed on theca interna cells and moderately on granulosa cells. GC: granulosa cells. TI: theca interna cells. Original magnification x120.

(Corner, 1956). For example, corpus luteum day 2 is the next day of ovulation.

**Indirect immunofluorescence histochemistry**

Immunohistochemistry proceeded as described (Fujiwara et al., 1993), with minor modifications. Each specimen was embedded in OCT compound (Tissue-Tec®; Miles Scientific, Naperville, IL, USA), snap-frozen in liquid nitrogen and stored at –80°C. Frozen tissues were sliced into 7 μm sections using a cryostat microtome (Cryocut 1800®; Reichert-Jung, Heidelberg, Germany), immediately air-dried on Neoplene® (Nissin EM, Tokyo, Japan)-coated glass slides. The slides were immediately examined or stored at –20°C until use. The slides were incubated with mAb 3B5 (10 μg/ml) or the anti-TNP mAb (10 μg/ml), for 30 min at room temperature. After washing in phosphate-buffered saline (PBS), they were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin (Ig) (diluted 1:50; Dakopatts A/S, Glostrup, Denmark), for 30 min at room temperature in the dark. The slides were washed, mounted with Perma Fluor Aqueous Mounting Medium® (Immunon, Pittsburgh, PA, USA), which reduces fluorescence fading, and examined under a fluorescence microscope (Nikon, Tokyo, Japan). The intensity of LH-R expression was graded in four scores from – to +++ based on fluorescence intensity (−, absence of staining; +, weak staining; ++, medium staining; ++++, intense staining). The samples were examined by two individuals within 6 h. If the judgement differed, the lower value was selected.

**Isolation of human granulosa cells**

Human granulosa cells were isolated from six patients, aged 24–37 years old, undergoing an in-vitro fertilization programme, as reported (Fukuoka et al., 1992). Briefly, they were hyperstimulated with human menopausal gonadotrophin (Organon Japan Co. Ltd, Tokyo, Japan) and a gonadotrophin releasing hormone analogue, buserelin acetate (Hoechst Japan Co. Ltd, Tokyo, Japan) until the follicles reached maturity (about 18–20 mm in diameter). Follicles were aspirated 36 h after the administration of HCG (5000 IU; i.m.; Mochida Pharmaceutical Co. Ltd, Osaka, Japan). The mean numbers of the aspirated follicles, obtained oocytes and fertilized oocytes were 11.6, 8.0 and 7.0, respectively. The suspension of granulosa cells was overlaid on Lymphocyte Separation Medium, Organon Teknika Corporation, Durham, NC, USA and centrifuged at 400 g for 30 min. The cells were collected from the interphase.
Figure 3. The expression of LH-R in a post-ovulatory follicle and a corpus luteum in mid-luteal phase detected by indirect immunofluorescence staining. **A-C**: a post-ovulatory follicle (corpus luteum day 2, the day after ovulation). **D-F**: a corpus luteum at day 6. **A** and **D**: haematoxylin and eosin staining. **B** and **E**: LH-R expression stained by 3B5 mAb. **C** and **F**: negative controls stained by anti-TNP mAb. **B**: LH-R was moderately expressed on both centrally- and peripherally-located luteinizing cells. **E**: large luteal cells intensely expressed LH-R. **CLC**: centrally-located luteinizing cells. **PLC**: peripherally-located luteinizing cells. **CC**: central cavity. **LL**: large luteal cells. **SL**: small luteal cells. Original magnification x120.

**Indirect immunofluorescence staining of isolated granulosa cells by 3B5 mAb**

The isolated human granulosa cells were washed in Hank’s balanced salt solution (HBSS) with 0.1% bovine serum albumin (BSA) and 0.1% NaN₃, sedimented by centrifugation and incubated with 5 µl mAb 3B5 (100 µg/ml) or the anti-TNP mAb (100 µg/ml) for 30 min at 4°C. After washing in HBSS, the cell pellet was incubated with FITC-conjugated rabbit anti-mouse Ig, for 30 min at 4°C in the dark. After washing in HBSS, the cells were resuspended in glycerin/PBS (1:1, v/v) and the stained cells were observed under a fluorescence microscope (Nikon).

**Flow cytometrical analysis of the effects of HCG or TNFα on human luteinizing granulosa cells cultured in serum-free medium**

The isolated human granulosa cells, as described above, were suspended (5.0 × 10⁵ cells/ml) in RPMI 1640 containing 10% FCS, 100 µg/ml kanamycin sulfate (Meiji Seika, Tokyo, Japan), then cultured on six-well plates (Becton Dickinson, Lincoln Park, NJ, USA) for 24 h. The following day (day 1), the medium was discarded to remove unattached cells and was replaced with serum-free DMEM/F12 (1:1, v/v, Gibco, Grand Island, NY, USA) medium without (control) or with HCG (10 IU/ml; Serono Japan, Tokyo, Japan), or TNFα (10 ng/ml; Daiichi Pure Chemicals, Tokyo, Japan). The cells were cultured for 3 days, during which the serum-free medium was changed and HCG or TNFα was added every day. On day 4, flow cytometrical analysis proceeded as described (Hattori et al., 1995). The plates were gently washed with PBS to remove the unattached cells, then incubated with FITC-conjugated rabbit anti-mouse Ig, for 30 min at 4°C in the dark. After washing in HBSS, the cell pellet was incubated with FITC-conjugated rabbit anti-mouse Ig, for 30 min at 4°C in the dark. After washing in HBSS, the cells were resuspended in the same solution and viable cells were analysed by flow cytometry (FACScan®, Becton Dickinson Immunocytometry Systems, Tokyo, Japan). In each group, the cut-off value was defined as the uppermost fluorescence.
intensity stained by the anti-TNP mAb, and the LH-R positivity rate was defined as the rate of the cells stained by mAb 3B5 with an intensity higher than the defined cut-off value. The mean fluorescence intensity was defined as the mean fluorescence intensity stained with mAb 3B5 minus that stained by the anti-TNP mAb.

Statistical analysis
The differences of positive rate and mean fluorescence intensity of LH-R expression on granulosa cells in flow cytometry were analysed by repeated one-way analysis of variance followed by the Scheffé F-test.

Results

Expression profiles of LH-R in human ovarian follicles

In a pre-antral follicle, which had three or four layers of granulosa cells, some thecal cells moderately expressed LH-R (Figure 1B). In small antral follicles <1 mm in diameter (n = 2), theca interna cells moderately expressed LH-R (Figure 1E). In growing follicles, 4–8 mm in diameter (n = 4), theca interna cells moderately expressed LH-R, but granulosa cells did not (Figure 2B). In leading follicles 12–15 mm in diameter (n = 4), granulosa cells weakly expressed LH-R, whereas theca interna cells moderately expressed it (data not shown). In pre-ovulatory follicles 18–20 mm in diameter (n = 4), LH-R was moderately or weakly expressed on granulosa cells and was expressed intensely or moderately on theca interna cells (Figure 2E). In all the examined follicles, immunoreactive LH-R was observed on the cell surface and cytoplasmic regions of theca interna cells and granulosa cells. In large follicles, very weak staining was sometimes observed in the nuclear region of stromal cells around theca interna cells. The expression of LH-R in follicles is summarized in Table I.

Table I. Fluorescence intensity scores of luteinizing hormone/human chorionic gonadotrophin (LH/HCG) receptor expression on granulosa and theca interna cells in antral follicles

<table>
<thead>
<tr>
<th>Diameter of follicles</th>
<th>LH/HCG receptor</th>
<th>granulosa cells</th>
<th>theca interna cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Preantral follicle (n = 1)</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>2. Antral follicles (n = 14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 mm (n = 2)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4–8 mm (n = 4)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12–15 mm (n = 4)</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>18–21 mm (n = 4)</td>
<td>+ + +</td>
<td>+ + +</td>
<td>++++</td>
</tr>
</tbody>
</table>

The intensity of the antigen expression was graded in four scores from - (absence of staining), ++ (weak staining), +++ (intense staining) based upon its fluorescence intensity. The scores were judged by two individuals. +++ means that some cells were moderately positive. n = number of follicles.

Expression profiles of LH-R in human corpus luteum of the menstrual cycle

In the early luteal phase (corpus luteum day 2–5, n = 9), LH-R was moderately or intensely expressed on centrally-located luteinizing granulosa cells, which are thought to differentiate to large luteal cells. LH-R was weakly or moderately expressed on peripherally-located luteinizing cells, which differentiate to small luteal cells (Figure 3B). In the mid-luteal phase (corpus luteum day 6–10, n = 10), LH-R was intensely or moderately expressed on the cell surface and cytoplasmic regions of large luteal cells (Figures 3E, 4B). On small luteal cells, LH-R was mainly expressed on cytoplasmic regions. In the corpus luteum at day 11–12 (n = 2), LH-R was weakly expressed on both luteal cells (data not shown). In the corpus luteum at day 13–14 (n = 2), LH-R was almost undetectable on both luteal cells (Figure 4E). In some corpora lutea, weak staining was observed in the nuclear region of stromal cells around the corpus luteum (Figure 4B).

Expression profiles of LH-R in human corpus luteum of early pregnancy

In the corpus luteum of early pregnancy at 6, 7 (n = 2), 9, 11, 14, and 15 weeks gestation, LH-R was expressed at various intensities (from weak to intense) on the cell surface and cytoplasmic regions of large luteal cells. In contrast, LH-R was weakly or moderately expressed on the nuclear membranes and cytoplasmic regions of small luteal cells (Figure 5B and E). The expression intensity of LH-R in the corpus luteum is summarized in Table II.

Table II. Fluorescence intensity scores of luteinizing hormone/human chorionic gonadotrophin (LH/HCG) receptor expression on granulosa/large theca/small luteal cells in corpora lutea

<table>
<thead>
<tr>
<th>LH/HCG receptor</th>
<th>G/LL</th>
<th>T/SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early luteal corpus luteum (n = 9)</td>
<td>+++(+++++)</td>
<td>-++</td>
</tr>
<tr>
<td>Mid-luteal corpus luteum (n = 10)</td>
<td>(++-)+++</td>
<td>++</td>
</tr>
<tr>
<td>Late luteal corpus luteum (n = 2)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regressing corpus luteum (n = 2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corpus luteum of pregnancy (n = 4)</td>
<td>+++++</td>
<td>+++</td>
</tr>
<tr>
<td>at 6–9 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus luteum of pregnancy (n = 3)</td>
<td>++++</td>
<td>-++</td>
</tr>
<tr>
<td>at 11–15 weeks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The intensity of the antigen expression score was graded as described in Table I. G/LL = granulosa/large luteal cells; T/SL = theca/small luteal cells. n = number of corpora lutea.

Expression of LH-R on the cell surface of isolated luteinizing granulosa cells

LH-R was moderately expressed on the cell surface region of luteinizing granulosa cells obtained from the patients undergoing in-vitro fertilization (Figure 6A).

Flow cytometrical analysis of the effects of HCG and TNFα on the expression of LH-R on cultured human luteinizing granulosa cells

The rate of the LH-R positive cells was 58.1 ± 19.5, 67.8 ± 17.4, 56.7 ± 20.6 % in the control, HCG-treated and TNFα-treated groups respectively (mean ± SD), and there was no significant difference (n = 6). However, the mean fluorescence intensity was 52.5 ± 19.4, 147.9 ± 29.6, 46.1 ± 10.6, respectively (mean ± SEM), and it was significantly higher in the HCG-treated groups than in the other two groups (P <
Figure 4. The expression of LH-R on corpus luteum at day 9 and day 14 detected by indirect immunofluorescence staining. A-C: a corpus luteum at day 9. D-F: a corpus luteum at day 14. A and D: haematoxylin and eosin staining. B and E: LH-R expression stained by 3B5 mAb. C and F: negative controls stained by anti-TNP mAb. B: LH-R was moderately expressed on large and small luteal cells. Weak staining was observed in some stromal cells (arrows). E: LH-R was almost undetectable in both luteal cells. LL: large luteal cells. SL: small luteal cells. Original magnification x120.

Discussion

In the growing follicles, 4–8 mm in diameter, LH-R was expressed on theca interna cells. This result is compatible with the finding demonstrated by Shima et al. (1987) using autoradiography and confirms the previous report that the theca interna layer is the main target site of LH in human follicles (Tsang et al., 1979). In this study, we showed that theca interna cells in pre-antral and small antral follicles, <1 mm in diameter, expressed immunoreactive LH-R. Shima et al. (1987) also showed that LH binding sites were present on the theca interna layer in small follicles. Hillier et al. (1980) demonstrated that human small follicles contain high concentrations of androgen. It has been reported that some human preantral follicles express steroidogenic enzymes on theca interna cells such as the cholesterol side chain cleaving enzyme, 3β-hydroxysteroid dehydrogenase, and 17α-hydroxylase (Suzuki et al., 1994). In addition, immunoreactive androgen receptors were reported to be expressed on human small antral follicles (Horie et al., 1992; Suzuki et al., 1994). Since androgen enhances the oestradiol production by granulosa cells androgen may affect early follicular development in a paracrine fashion (Daniel and Armstrong, 1980; Hillier and De Zwart, 1981). The detection of immunoreactive LH-R on theca interna cells in pre-antral and small antral follicles suggests the involvement of LH in the production of androgen in these follicles and supports the involvement of an endocrine system in early follicular development in the human ovary.

In leading follicles 12–15 mm in diameter, although no LH binding sites were reportedly detected on granulosa cells (Shima et al., 1987), we observed the expression of immunoreactive LH-R on granulosa cells in this study. This suggests a physiological role of LH on the function of granulosa cells in leading follicles (Hillier, 1994). In pre-ovulatory follicles, granulosa cells are considered able to respond to the signal of
Figure 5. The expression of LH-R in corpus luteum of pregnancy at 6 and 11 weeks of gestation detected by indirect immunofluorescence staining. A-C: a corpus luteum of pregnancy at 6 weeks of gestation. D-F: a corpus luteum at 11 weeks of gestation. A and D: haematoxylin and eosin staining. B and E: LH-R expression stained by 3B5 mAb. C and F: negative control stained by anti-TNP mAb. B: LH-R was expressed moderately on the cell surface regions of large luteal cells, and weakly on the nuclear membranes of small luteal cells. E: LH-R was detected mainly in cytoplasmic regions in both luteal cells. LL: large luteal cells. SL: small luteal cells. Original magnification x120.

mid-cycle LH surge and start to luteinize. In preparation, granulosa cells must have acquired an adequate amount of LH-R on their cell surface. This study confirmed the increase of LH-R expression on granulosa cells before ovulation. We sometimes observed weak staining on the nuclear regions of the stromal cells around follicles of the late follicular phase and around corpus luteum of the menstrual cycle, suggesting that these cells may respond to LH/HCG although LH-R was not detectable on the cell surface.

The immunofluorescence staining of isolated granulosa cells demonstrated the presence of immunoreactive LH-R on the cell surface of luteinizing granulosa cells and immunohistochemistry revealed that luteinizing granulosa cells increased immunoreactive LH-R expression during the corpus luteum formation. Since HCG enhances progesterone production by luteinizing granulosa cells in vitro (Emi et al., 1991), endogenous LH may stimulate progesterone production by luteinizing granulosa cells through LH-R during the corpus luteum formation. Flow cytometrical analysis showed that HCG administered for 3 days enhanced the mean fluorescent intensity of immunoreactive LH-R and did not affect the rate of the LH-R-positive cells. In various species, internalization of LH-R and down-regulation of synthesis of LH-R by LH/HCG have been observed. Several researchers have shown that the mRNA of LH-R in rat granulosa cells decreases after an injection of an ovulatory dose of HCG, and subsequently increased spontaneously (Segaloff et al., 1990; Camp et al., 1991; Peng et al., 1991). The present study suggests that HCG enhanced or maintained the number of LH-R on the cell surface of human luteinizing granulosa cells in a long term culture. Since luteinizing granulosa cells in the early corpus luteum express high levels of immunoreactive LH-R, endogenous LH secreted from the pituitary gland may enhance or maintain LH-R on luteinizing granulosa cells during corpus luteum formation. In addition to the luteotrophic function of LH/HCG, we proposed that cytokines affect the maturation of the corpus luteum during corpus luteum formation. We have shown that large luteal cells express human leukocyte antigen (HLA)-DR, leukocyte functional antigen (LFA)-3, and dipeptidyl peptidase IV (DPP-IV).
Figure 6. The expression of LH-R on the cell surface of isolated human luteinizing granulosa cells detected by indirect immunofluorescence staining. A: LH-R is detected on the cell surface region of granulosa cells by 3B5 mAb. B: a phase contrast picture of A. C: negative control stained by anti-TNP mAb. D: a phase contrast picture of C. Original magnification x240.

Figure 7. Histograms of flow cytometry of cultured human luteinizing granulosa cells. Human granulosa cells were cultured for 3 days in serum-free medium without (control), or with HCG (10 IU/ml), or with TNFα (10 ng/ml). On day 4, LH-R expression on granulosa cells was examined by flow cytometry. The anti-TNP mAb was used as negative control. In the HCG-treated group, the LH-R histogram showed that the mean fluorescence intensity was higher than those in the other groups.

(Fujiwara et al., 1992; Fujiwara et al., 1993; Hattori et al., 1995). The expression of these cell surface molecules is augmented during corpus luteum formation in vivo. Flow cytometrical analysis revealed that TNFα, but not HCG, increases the expression of LFA-3 on cultured luteinizing granulosa cells, and that TNFα and interleukin-1α, but not HCG, enhance DPP-IV expression on granulosa cells (Fujiwara et al., 1994; Hattori et al., 1995). These results imply that the maturation of the early corpus luteum is regulated not only by pituitary LH but also by local factors such as cytokines. Therefore we examined the effect of TNFα on the LH-R expression on human luteinizing granulosa cells in this study. In contrast to the effect of HCG, TNFα did not affect LH-R expression on luteinizing granulosa cells. This supports the theory that HCG and cytokines play differential stimulatory roles in the maturation of luteinizing granulosa cells during corpus luteum formation (Fujiwara et al., 1994; Hattori et al., 1995).

In mid-luteal phase, the highest level of immunoreactive LH-

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in vivo


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rulation during maternal recognition of pregnancy. Taken together with the flow regulation during corpus luteum of the menstrual cycle. Corner, G.W. (1956) The histological dating of the human corpus luteum of pregnancy. We are also grateful to Mr Daniel Mrozek for

References


Ohara R was expressed on the cell surface region of large luteal cells, compatible with the histological results shown by autoradiography and mRNA studies (Shima et al., 1987; Nishimori et al., 1995). It also agrees with the results of binding assays using human corpus luteum homogenates (Rao et al., 1977; McNeilly et al., 1980; Rajaniemi et al., 1981; Yamoto et al., 1986). We have previously reported that a preparation rich in large luteal cells isolated from human corpus luteum secreted more progesterone than a preparation rich in small luteal cells (Ohara et al., 1987). On the other hand, more testosterone and androstenedione were secreted by preparations rich in small luteal cells than large luteal cells. Since the secretion of these two androgens and progesterone were significantly augmented by HCG in preparations rich in small luteal cells, we speculated that small luteal cells secreted androgen stimulated by endogenous LH in the menstrual cycle. In contrast, there was no significant augmentation of progesterone secretion by large luteal cells in response to FSH or HCG exposure in the culture for 24 h, probably because large luteal cells in the mature corpus luteum had already reached their maximum ability to produce progesterone and did not respond to HCG. If so, the physiological significance of high expression of LH-R on large luteal cells in mature corpus luteum is not the stimulation of progesterone production, but the reception of the embryonic signal for differentiation into the corpus luteum of pregnancy. It is strongly suggested that the main target cells for HCG, the embryonic signal, are large luteal cells and that HCG prevents the corpus luteum from regression or promotes its differentiation into the corpus luteum of pregnancy through a direct influence on large luteal cells.

In corpus luteum of early pregnancy, LH-R was also expressed on large luteal cells, whereas it was not in regressing corpus luteum of the menstrual cycle. Taken together with the flow cytometry results, it can be speculated that when pregnancy occurs, HCG secreted by the embryo maintains the expression of LH-R and the response of large luteal cells to HCG. This position also agrees with the finding that exogenous HCG does not down-regulate LH-R expression in the corpus luteum of the mid-luteal phase (Duncan et al., 1996). In this regard, the clinical application of HCG in luteal support therapy for infertile women may effectively enhance LH-R expression in the corpus luteum to augment responsiveness to the HCG signal from the embryo.

The luteal-placental shift of progesterone production is assumed to occur at 7–9 weeks of gestation (Yen, 1991). In the corpus luteum of pregnancy, immunoreactive LH-R was also expressed on large luteal cells even after the shift. There are some reports that the human corpus luteum during term is still functional (Nelson et al., 1958; Adams and Hertig, 1969; Derkx et al., 1987). Corpus luteum after the luteal-placental shift may still respond to HCG signal and play some roles in the maintenance of pregnancy.

In conclusion, LH-R is expressed mainly on large luteal cells during corpus luteum formation and early pregnancy in vivo. An in-vitro study suggested that HCG enhances or at least maintains the expression of its own receptors on granulosa cells/large luteal cells during corpus luteum formation. These findings suggest that large luteal cells are the main target cells of the HCG signal from the embryo during the differentiation of corpus luteum in the menstrual cycle to that of pregnancy.

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