Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation

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Human luteal cells have been reported to express human leukocyte antigen-DR and lymphocyte functional antigen-3 on the cell surface, suggesting physiological interaction between luteal cells and T-lymphocytes through the menstrual cycle into early pregnancy. To elucidate the role of peripheral lymphocytes on corpus luteum differentiation, the effects of peripheral blood mononuclear cells (PBMC) on steroidogenesis by luteal cells was investigated. The production of Th-2 cytokines such as interleukin (IL)-4 and IL-10 by the co-cultured cells was also examined, and the effects of these cytokines on progesterone production by luteal cells were investigated. Corpora lutea were obtained from eight non-pregnant women in the luteal phase and five women in early pregnancy for luteal cell culture. PBMC were isolated from unrelated women in the follicular phase, secretory phase, and early pregnancy. After coculture with allogenic PBMC for 48 h, progesterone production was significantly enhanced by PBMC from the secretory phase and early pregnancy in the non-pregnant luteal cell culture. In the pregnant luteal cell culture, a significant increase in progesterone production was also observed by the co-culture with PBMC from women in early pregnancy, showing that PBMC have a luteotropic effect. The stimulatory effects of PBMC were also observed in co-culture conditions which prevented direct cell-to-cell interaction with luteal cells, showing the minor influence of mixed lymphocyte reaction. By co-culture with PBMC, the production of IL-10, but not IL-4, was significantly augmented in luteal cell culture derived from non-pregnant women, whereas the production of both IL-4 and IL-10 was significantly enhanced in the luteal cell culture derived from pregnant women. Moreover, IL-4 and IL-10 promoted progesterone production by cultured luteal cells, especially in the luteal cell culture derived from corpora lutea of early pregnancy. These findings indicate that PBMC stimulate progesterone production by luteal cells and suggest the involvement of PBMC in corpus luteum function and differentiation probably via the Th-2-type lymphocytes.

Key words: corpus luteum/interleukin-4/interleukin-10/luteal cells/peripheral blood lymphocyte

Introduction

The role of immune cells in the ovary is a subject of considerable interest. More specifically, the effects of cytokines on steroidogenesis in ovarian cells have been investigated, mainly utilizing cultured cells in vitro (Fukuoka et al., 1988; Adashi, 1990; Mori, 1990). We reported that cytokines such as interleukin-1α (IL-1α), tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) modulated progesterone synthesis in human luteal cells (Fukuoka et al., 1992) and that these cytokines regulated the expression of differentiation-related molecules which are specifically expressed on luteal cells during corpora lutea (CL) formation and transition to CL of pregnancy (Fujiwara et al., 1994; Hattori et al., 1995; Fujiwara et al., 1996). These cytokines are known to be produced by leukocytes, such as macrophages, lymphocytes and neutrophils, that infiltrate CL (Wang et al., 1992). Furthermore, we reported that an undefined soluble factor(s) derived from peripheral lymphocytes stimulated steroidogenesis by human luteinizing granulosa cells (Emi et al., 1991). Based on the above findings, it is proposed that immune cells are involved physiologically in the generation processes of CL.

In general, it has been accepted that the differentiation and maturation of CL are regulated endocrinologically by gonadotrophins from the pituitary gland in the menstrual cycle or human chorionic gonadotrophin (HCG) from villous trophoblasts in the early stage of pregnancy (Yen, 1991). However, the precise mechanism as to how CL fail to degenerate when pregnancy occurs is still unknown. To resolve this problem, the concept of a dynamic balance between the two counteracting factors, called luteotrophin and luteolysin, was proposed where luteinizing hormone (LH) and HCG represented the former and prostaglandin F2α (PGF2α) represented the latter (Auletta and Flint, 1988; Niswender et al., 1994).

We previously demonstrated that human luteal cells in CL of early pregnancy express human leukocyte antigen (HLA)-DR and lymphocyte functional antigen (LFA)-3, suggesting a physiological interaction between lymphocytes and luteal cells during early pregnancy (Fujiwara et al., 1993a; Hattori et al., 1995). We postulated that immune cells in early pregnancy possess information on the presence of the embryo, transmit it to the CL and regulate CL differentiation (Hattori et al., 1995). In mice, i.v. administration of splenocytes obtained from early pregnancy, but not pseudopregnancy, day 4 and dioestrous day 2 mice, enhanced implantation rates in the female.
Materials and methods

Isolation of luteal cells
Corpora lutea (CL) were obtained from eight non-pregnant women (CL day 10–12) aged 33 to 42 years at abdominal hysterectomy for uterine myoma, and from five pregnant women (7–10 weeks of gestation) aged 28 to 36 years at laparotomy for ectopic pregnancy and abdominal hysterec¬tomy for uterine myoma. The purpose of the present study was explained to the patients in all cases, and informed consent was obtained from them. None of the patients were on hormone therapy before the operation. We determined the postovulatory day based on basal body temperature and confirmed it by histological dating of CL according to Corner (1956). In this work, the term ‘CL day’ was used according to his definition. For example, CL day 2 is the next day of ovulation.

Isolation of luteal cells was performed as described previously with minor modification (Fujiwara et al., 1996). Briefly, the CL were separated from connective tissue, then minced with scissors and incubated in RPMI 1640 (Flow Labs., Irvine, Scotland) containing 5% fetal calf serum (FCS; Flow Laboratories, McLean, VA, USA), 0.2% type I collagenase (Sigma Chemical Co., St. Louis, MO, USA) and 0.005% deoxyribonuclease I (Sigma) at 37°C for 1 h. After standing the tube for 1 min to let debris sink, the cell suspension was overlaid on Ficoll–Hypaque (Nacalai Tesque, Kyoto, Japan) and centrifuged at 20 min for 400 g. The cells at the interphase were washed twice and resuspended in RPMI 1640 with 10% FCS, penicillin G (50 µg/ml; Sigma) and streptomycin sulphate (50 µg/ml; Sigma). The Trypan Blue exclusion test revealed that cell viability ranged from 80 to 90%. The cell suspension was adjusted to 2×10^5 cells/ml and was inoculated on a 48-well plate coated with human type IV collagen (Becton Dickinson Labware, Bedford, MA, USA).

Preparation of PBMC
PBMC were prepared as described previously (Emi et al., 1991). Volunteers were recruited from healthy non-pregnant women (follicular phase, n = 13; secretory phase, n = 13) with a regular menstrual cycle and not on hormone therapy and healthy pregnant women (7–10 weeks of gestation, n = 13) with a definite duration of gestation. PBMC were isolated from 16 ml of venous blood by means of centrifugation with Ficoll–Hypaque. After centrifugation, PBMC were collected from the interphase layer and washed four times with RPMI 1640. To exclude the effect of contamination with soluble factors derived from serum, the final suspended medium of each patient’s PBMC was mixed and was used as a common suspension medium.

Co-culture of luteal cells and PBMC
The medium was replaced 12 h after the start of luteal cell culture and, at the same time, the prepared PBMC were added. Each well was adjusted to contain 2.0×10^5 luteal cells/ml with or without 5.0×10^5 PBMC/ml in RPMI 1640 medium with 10% FCS, penicillin G (50 µg/ml) and streptomycin sulphate (50 µg/ml). To the control wells (luteal cells without PBMC), the same volume of a common suspension medium was added. Thereafter, the cells were co-cultured in triplicate at 37°C for 48 h in a humidified atmosphere of 5% CO2 in the air.

In some experiments (n = 4 for luteal cell culture from non-pregnant CL, and n = 2 from pregnant CL), after the initial 12-h culture of luteal cells, a basket-type of culture-well unit (Intercell, Kurabo, Tokyo, Japan) was put in each well of the culture plates. Then, PBMC were inoculated within the intercell and were co-cultured with luteal cells for 48 h. This intercell prevented any direct interaction of PBMC and luteal cells because of the micropore membrane located in the bottom of the intercell, which could only transmit soluble factors such as cytokines.

Sometimes luteal cells (2.0×10^5/ml) were cultured in triplicate without PBMC, in the presence or absence of recombinant IL-4 (10 ng/ml; Ono Pharmaceutical Co. Ltd, Osaka, Japan) and recombinant IL-10 (10 ng/ml; Gibco BRL, Grand Island, NY, USA) or HCG (1 IU/ml, Mochida Pharmaceutical Co. Ltd, Osaka, Japan) for 48 h (n = 6 for luteal cell culture from non-pregnant CL; n = 4 from pregnant CL).

At the end of each culture, the medium was collected and kept frozen at −20°C until assay for progesterone or cytokines.

Determination of progesterone and cytokines
Progesterone was measured as described previously by radioimmunoassay with a commercial kit (Daichi Radio Isotope Research Inc., Tokyo, Japan) (Fujiwara et al., 1997). The concentrations of IL-4 and IL-10 were determined by immunoenzymometric assay kits (IL-4 EASIA® and IL-10 EASIA®; Medgenix Diagnostics, Fleurus, Belgium). The minimal detectable concentration was 2 pg/ml for IL-4 and 1 pg/ml for IL-10, respectively.

Statistics
The concentrations of progesterone and cytokines are shown as means ± SEM. The difference of progesterone production was analysed by the one-way analysis of variance, followed by Scheffé’s F-test. The difference between the cytokine concentration in the co-culture and the sum of cytokine concentration in each PBMC culture and luteal cell culture was analysed by the two-tailed paired t-test. The difference was considered to be significant at P < 0.05.

Results

Effect of PBMC on progesterone secretion by luteal cells
Progesterone secretion by luteal cells obtained from non-pregnant women was significantly enhanced by co-culture with
PBMC isolated from women in the luteal phase and early pregnancy \((n = 8, P < 0.05\) and \(P < 0.01\), respectively), whereas the significant augmentation of progesterone production was not observed in co-culture with PBMC from the women in the follicular phase (Figure 1). The stimulatory effect of PBMC on progesterone production was significantly higher in the PBMC from early pregnant women than that from women in the follicular phase.

In the luteal cell culture isolated from CL in early pregnant women, progesterone production was also significantly enhanced by co-culture with PBMC derived from early pregnant women \((n = 5, P < 0.01)\). On the other hand, the intensity of the stimulatory effect in the PBMC derived from non-pregnant women was not significant, although about a 1.5-fold increase of progesterone production was observed (Figure 2).

In the luteal cell culture obtained from non-pregnant women using intercells, progesterone secretion was also enhanced by co-culture with PBMC isolated from the women in the luteal phase and early pregnancy \((n = 4)\). No significant difference in the intensity of increase in progesterone production was observed between the groups co-cultured with and without intercells (Figure 3). A similar effect of PBMC was also observed in the luteal culture derived from pregnant women in spite of the use of intercells \((n = 2, \text{data not shown})\).

**Cytokine concentrations in culture supernatant**

In the culture medium of luteal cells co-cultured with PBMC, IL-4 and IL-10 could be detected by immunoenzymometric assay. The concentrations of these cytokines were also measured in the supernatant when luteal cells or PBMC were cultured alone.

In the luteal cell culture isolated from non-pregnant CL, the concentration of IL-10, but not IL-4, in the co-culture with PBMC was significantly higher than that of the sum of each PBMC culture and luteal cell culture (Table I). This increase was prominent in the co-culture with PBMC from early pregnant women.

In the luteal cell culture isolated from pregnant CL, the concentration of IL-10 in the co-culture with PBMC was significantly higher than that of the sum of each PBMC culture and luteal cell culture. Additionally, the concentration of IL-4 was also elevated in the co-culture with PBMC from early pregnant women (Table 1).

These elevations of cytokine production were not observed in co-culture using intercells (data not shown).
In the luteal cell culture derived from non-pregnant women, all the interleukin (IL)-4, IL-10, and human chorionic gonadotrophin (HCG) significantly promoted progesterone production. **P < 0.01. CTR = controls.

**Figure 4.** Effect of cytokines on progesterone secretion by luteal cells derived from non-pregnant women. Significant enhancement of progesterone production by the luteal cells was observed in the groups receiving interleukin (IL)-4 and IL-10 administration. The addition of human chorionic gonadotrophin (HCG) also stimulated progesterone production, but its effect was not significant. *P < 0.05. CTR = controls.

Table I. Cytokine production in co-culture of luteal cells with peripheral blood mononuclear cells (PBMC)

<table>
<thead>
<tr>
<th>Cytokines (pg/ml)</th>
<th>IL-4</th>
<th>IL-10</th>
</tr>
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<tbody>
<tr>
<td>Non-pregnant PBMC</td>
<td>8.02 ± 2.11</td>
<td>15.8 ± 3.21</td>
</tr>
<tr>
<td>Pregnant PBMC</td>
<td>9.86 ± 4.13</td>
<td>21.5 ± 3.55</td>
</tr>
<tr>
<td>Membratal CL</td>
<td>31.2 ± 5.88</td>
<td>222 ± 37.6</td>
</tr>
<tr>
<td>Luteal cells 4.86 ± 2.37</td>
<td>12.3 ± 5.16</td>
<td>340 ± 67.9</td>
</tr>
<tr>
<td>+ Non-pregnant PBMC</td>
<td>3.33 ± 1.21</td>
<td>222 ± 37.6</td>
</tr>
<tr>
<td>+ Pregnant PBMC</td>
<td>176 ± 11.3</td>
<td>376 ± 42.3</td>
</tr>
<tr>
<td>Pregnancy CL</td>
<td>24.4 ± 7.22</td>
<td>158 ± 28.0</td>
</tr>
<tr>
<td>Luteal cells 5.31 ± 2.37</td>
<td>32.8 ± 13.5</td>
<td>158 ± 28.0</td>
</tr>
<tr>
<td>+ Non-pregnant PBMC</td>
<td>32.8 ± 13.5</td>
<td>158 ± 28.0</td>
</tr>
<tr>
<td>+ Pregnant PBMC</td>
<td>176 ± 21.9</td>
<td>376 ± 42.3</td>
</tr>
</tbody>
</table>

†Cytokine concentration in the co-culture of PBMC and luteal cells was significantly higher than the sum of cytokine concentration in each PBMC culture and luteal cell culture separately (**P < 0.05; **P < 0.01).

‡Cytokine concentration in the co-culture of PBMC and luteal cells was significantly lower than the sum of cytokine concentration in each PBMC culture and luteal cell culture separately (**P < 0.01).

**Effect of cytokines on progesterone secretion by luteal cells**

In the luteal cell culture derived from non-pregnant women, IL-4, IL-10, and HCG significantly promoted progesterone production (Figure 4).

Significant enhancement of progesterone production by the luteal cells derived from pregnant women was also observed in the groups receiving IL-4 and IL-10 administration (Figure 5). The addition of HCG also stimulated progesterone production, but its effect was not significant.

**Discussion**

During the normal menstrual cycle, the CL degenerate after a life span of 14 days. When a woman becomes pregnant, degeneration is retarded at least until the so-called luteoplacental shift (between the 7–9 weeks of pregnancy), during which the CL of pregnancy are considered to play an important role in maintaining pregnancy. Mechanisms underlying the generation and degeneration of menstrual CL, and its transition to the CL of pregnancy are still uncertain. In general, it has been accepted that HCG secreted from conceptus plays an important role in the transformation of menstrual CL into pregnancy CL (Yen, 1991). Recently, we showed immunohistochemically that, in CL of early pregnancy, immunoreactive LH/HCG-receptor is expressed on large luteal cells, whereas it is hardly expressed in regressing CL of the menstrual cycle (Takao et al., 1997). We also demonstrated that, in regressing CL, the expression of low-density lipoprotein (LDL) receptor is very weak and the uptake of LDL is hardly detected. In contrast, large luteal cells in CL of early pregnancy express immunoreactive LDL receptor, and the uptake of LDL was detected in these cells (Yamada et al., 1998). These findings suggest that the reduction of steroidogenesis in regressing CL is partially due to the attenuated expression of LH/HCG and LDL receptors on luteal cells, but it is still unknown how and what regulates the expression of these molecules when conceptus is present in the reproductive tract.

Although human CL in the term period of pregnancy are considered to be still functional (Nelson et al., 1958; Adams and Hertig, 1969; Derkx et al., 1987), HCG alone cannot induce progesterone secretion from the normal CL for more than a few weeks, and how progesterone secretion is regulated beyond that time is unknown (Weiss et al., 1977; Quagliarello et al., 1980). In 7 weeks of gestation, more than 15 ng/ml in the serum progesterone is estimated to be the production of CL of pregnancy, and the removal of CL in this period led to abrupt reduction of progesterone in the serum to induce abortion (Csapo et al., 1973). In addition, the HCG-induced relaxin production in the menstrual CL cannot be maintained beyond a few weeks by HCG stimulation alone (Quagliarello et al., 1980), although relaxin and progesterone production by CL of pregnancy continues until delivery (Weiss et al., 1977). On the other hand, it was reported that steroidogenesis by CL in ectopic pregnancy was reduced in spite of equal values of immunoreactive HCG as compared with normal pregnancy (Hubinont et al., 1987) and that the lower serum steroid level
in women with ectopic pregnancy cannot be explained by altered HCG bioactivity (Norman et al., 1988). Thus, investigators speculated that the synthesis of a factor other than HCG produced by the embryo or by the endometrium in response to implantation may be necessary to control CL function (Norman et al., 1988; Johnson et al., 1993a,b).

Recently, it has been proposed that the immune system plays a role in ovarian function (Adashi, 1990). Numerous examples of their involvement in luteolysis have been demonstrated (Auletta and Flint, 1988; Townsend et al., 1996). However, it is also unknown why the CL can escape from the attack by immune cells during pregnancy. Two mechanisms can be proposed for these different modes of interaction between luteal cells and immune cells in the regressing and pregnant CL. One is the functional changes of regressing or pregnant CL against the immune system during their differentiation process. In fact, we reported that the CL of pregnancy are in a further differentiated stage judging from the expression profiles of differentiation-related molecules, such as aminopeptidase-N and integrins on luteal cells (Fujiwara et al., 1992, 1993a; Honda et al., 1995). The other explanation is the alteration of immune cell function especially during pregnancy. To support the former possibility, it was reported that bovine regressing CL increased the expression of major histocompatibility complex (MHC) class II antigen, which is induced by interferon-γ, and that luteal cells elicit MHC class II-dependent T-lymphocyte proliferation, suggesting the involvement of MHC class II antigen in the process of luteolysis (Fairchild and Pate, 1989; Fairchild et al., 1991; Petroff et al., 1997). However, we observed that human CL of pregnancy also highly express HLA-DR as well as regressing CL (Fujiwara et al., 1993b). Furthermore, both CL in the menstrual cycle and early pregnancy expressed LFA-3, which is a ligand for the CD2 molecule on the T-lymphocytes, suggesting a physiological interaction between luteal cells and peripheral lymphocytes during early pregnancy (Hattori et al., 1995). Thus, according to the latter possibility described above, we postulated that immune cells in early pregnancy possess information on the presence of the embryo and play a role in the regulation of CL function by failing to attack CL (Fujiwara et al., 1993b; Hattori et al., 1995; Mori et al., 1995).

This study showed that co-culture with PBMC derived from women in the luteal phase and early pregnancy stimulated progesterone production by luteal cells, demonstrating a luteotropic action of these PBMC. Conversely, the stimulatory effect was not observed in PBMC obtained from women in the follicular phase. Since the PBMC-suspended media were mixed and prepared to be common among the groups at the start of the culture, this difference is thought to be caused not by soluble substances in the serum, but by each PBMC function itself. Thus, the luteotropic action of PBMC is dependent on the stage of menstrual cycle and pregnancy. For interpretation of these results, it should be taken into account that PBMC used in our study were obtained from a different individual and may not precisely represent the in-vivo nature of lymphocytes infiltrating the CL. The luteal cell culture contains the donor’s own immune cells which are residual in CL. Therefore, coculture may evoke non-specific immunoreaction, that is a mixed lymphocyte reaction. However, co-culture using intercells, which prevented luteal cells from direct interaction with PBMC, also led to a similar luteotropic response by luteal cells as observed in co-culture without intercells, implying the minor influence of mixed lymphocyte reaction. These results also showed that the luteotropic effect of PBMC does not necessarily require direct cell–cell interaction and suggest that the cross-talk between luteal cells and PBMC by soluble factors is responsible for the increase in progesterone production.

In luteal cell cultures derived from CL of early pregnancy, the luteotropic effect of PBMC was evident in PBMC obtained from early pregnant women. This strongly supports the concept that peripheral immune cells receive information on the presence of the embryo around implantation and thereafter, and systemically transmit it to the ovary to facilitate transformation of menstrual CL into pregnancy CL or to maintain the CL function. Kratzer and Taylor (1990) showed that no additional soluble factors other than HCG, which are involved in the CL regulation, exist in the sera judging from their bioassay system and concluded that CL function is primarily regulated by the rate of change of HCG production. However, they did not take account of the presence of immune cells in the sera. The immune system has elaborate networks which can transmit large amounts of specific information all over the body. The success of fertilization and implantation is one of the most important pieces of information for women who must prepare for the following changes in the function of various organs during pregnancy. The most essential point of our hypothesis is that the cells, but not hormones, directly and systemically transmit specific information of the presence of the conceptus into the various organs, including the ovary. This mechanism can explain progesterone secretion by CL during pregnancy and may also provide a new insight into the regulatory system for functional alteration in maternal organs.

In the co-culture of luteal cells with PBMC, IL-4 and IL-10 were detected in the culture media. When PBMC were co-cultured with the luteal cells isolated from menstrual CL, the production of IL-10, but not IL-4, was significantly augmented, whereas the production of both IL-4 and IL-10 was significantly increased in the co-culture of luteal cells from pregnant women with PBMC from early pregnant women. These results suggest that some immunological activation took place during the interaction between luteal cells and PBMC, and that the properties of elicited activations are different among the groups according to the pregnant or non-pregnant status. On the other hand, these increases in cytokine production were not observed in the co-culture using intercells. This indicates that cell–cell interaction is necessary for the immunological activation described above. Although the influence of mixed lymphocyte reaction should not be excluded to interpret the data of cytokine production, it is suggested that the physiological activation of PBMC may occur in different fashions, according to the pregnant or non-pregnant status, when they are interacted with luteal cells.

To estimate the role of cytokines on luteal function, we examined the effects of IL-4 and IL-10 on the production of progesterone by luteal cells. In the luteal cell culture derived from pregnant women, the high secretion of progesterone was
observed in the groups receiving IL-4 and IL-10 administration as well as the HCG-treated group. Helper T-lymphocytes that play a major role in immune surveillance can be classified into Th-1-type and Th-2-type cells: the former cells produce IL-2, IFN-γ, TNF-α, and IL-12, while the latter cells produce IL-4, IL-5, IL-6, and IL-10. Th-1-type cells are involved in delayed hypersensitivity, while Th-2-type cells are involved in the production of antibody (Kelso, 1995). This study demonstrated that co-culture of luteal cells with PBMC induced production of IL-4 and IL-10 resulting in luteotropic activity, which was more evident when luteal cells and PBMC from pregnant women were co-cultured. Since the co-culture of luteal cells with PBMC using intercells also augmented progesterone production, in spite of the lack of elevated production IL-4 and IL-10, soluble factors other than IL-4 and IL-10 may be also involved in the stimulatory effect of PBMC. In this study, the direct interaction of luteal cells and PBMC from pregnant women elicited considerable elevation of IL-4 production as well as IL-10. Furthermore, the elevation of IL-4 production in the co-culture was evident when both luteal cells and PBMC were derived from pregnant women, suggesting that Th-2-type cells are deeply involved in the stimulatory effect of progesterone production by pregnant luteal cells. Thus, at present, we speculate that Th-2-type cells play a role in transformation of menstrual CL into pregnancy CL and in the maintenance of pregnancy CL.

In conclusion, this study showed that human PBMC, especially in early pregnancy, have a luteotropic effect on CL, providing evidence which supports the concept that peripheral immune cells receive information on the presence of the embryo and systemically transmit it to the ovary to facilitate the function of pregnancy CL. This study also suggested the involvement of Th-2-type cells in the luteotropic effect of PBMC. Since this hypothesis can be extended and applied to other organs, further clarification of this issue will contribute to understanding the mechanism of an appropriate adaptation of various organs for pregnancy.

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References
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