The human corpus luteum: reduction in macrophages during simulated maternal recognition of pregnancy

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It has been shown that immune cells, particularly macrophages, accumulate in the corpus luteum during luteolysis. This study aimed to investigate the effect of maternal recognition of pregnancy on the localization and numbers of macrophages in the human corpus luteum. Corpora lutea (n = 12) were obtained from normally cycling women at the time of hysterectomy and were dated on the basis of serial urinary luteinizing hormone (LH) estimation. In addition, corpora lutea (n = 4) were collected from women who had received daily doubling doses of human chorionic gonadotrophin (HCG) to mimic the hormonal changes of early pregnancy. Macrophages were localized by immunohistochemistry using an anti-CD68 antibody. Steroidogenic cells, steroidogenic cells of thecal origin and endothelial cells were identified on serial sections by immunohistochemistry for 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase and von Willebrand factor, respectively. The luteal cells capable of responding directly to HCG were identified by isotopic in-situ hybridization for messenger RNA encoding LH/HCG receptors. Macrophages were localized primarily to the vascular connective tissue and theca–lutein areas of the corpus luteum, although some were found in the granulosa–lutein cell layer. Macrophage numbers increased throughout the luteal phase to a maximum in the late-luteal phase (P < 0.05). Luteal ‘rescue’ with HCG was associated with a marked reduction in the numbers of tissue macrophages when compared with those of the late-luteal phase (P < 0.001). One of the effects of HCG during maternal recognition of pregnancy is to prevent the normal influx of macrophages into the corpus luteum. As LH/HCG receptors localized to the steroidogenic cells, this implies a fundamental role for steroidogenic cell products in the control of macrophage influx into the human corpus luteum.

Key words: corpus luteum/macrophages/human chorionic gonadotrophin/pregnancy/luteolysis

Introduction

In a non-conception cycle, the primate corpus luteum undergoes luteolysis with a loss of functional and structural integrity. The molecular events involved in luteolysis and how they are prevented by exposure to human chorionic gonadotrophin (HCG) during maternal recognition of pregnancy remain unclear (Behrman et al., 1993). One feature of luteolysis, however, is the marked accumulation of immune cells in the corpus luteum. This increase in the number of immune cells has been reported in a variety of species, including rodents (Brännström et al., 1994a), rabbits (Naftalin et al., 1997), ruminants (Murdoch, 1987; Lei et al., 1991), and women (Wang et al., 1992a; Best et al., 1996; Takaya et al., 1997). As these species use disparate mechanisms to control their corpora lutea (Auletta and Flint, 1988), this common increase implies a fundamental role for immune cells, or their cytokine products (Brännström and Norman, 1993), in the luteolytic process.

The main immune cell to be localized in the human corpus luteum during luteolysis is the macrophage (Wang et al., 1992a; Brännström et al., 1994b; Best et al., 1996). However, macrophage products have been shown to have both positive and negative effects on progesterone secretion. Macrophage products have been reported to have pro-steroidogenic effects in cell culture (Kirsch et al., 1981, 1983; Halme et al., 1985). In addition, in the early stages of luteal function, macrophages are thought to have primarily luteotropic effects (Brännström and Norman, 1993). Other macrophage products, however, such as tumour necrosis factor α (TNFα), prostaglandin F2α (PGF2α), reactive oxygen species and nitric oxide, have been shown to have negative effects on steroidogenesis (Benyo and Pate, 1992; Grusenmeyer and Pate, 1992; Van Voorhis et al., 1994; Vega et al., 1995; Kato et al., 1997). Whereas macrophages are likely to be involved in the phagocytic clearance of cell debris (Paavola, 1979; Takaya et al., 1997) after functional luteolysis, it is still not clear what role tissue macrophages have in the functional corpus luteum.

We postulated that the primary role of macrophages in the human corpus luteum was luteolytic, rather than luteotropic, and that macrophage numbers would not increase in the ‘rescued’ corpus luteum of early pregnancy. To test this hypothesis, we investigated the number of macrophages in the human corpus luteum throughout the functional luteal phase by immunohistochemistry. We compared the numbers of macrophages in corpora lutea in the late-luteal phase, where progesterone output is falling, with corpora lutea ‘rescued’ with exogenous HCG, where progesterone output is increasing. We also postulated that any increase in macrophages within the corpus luteum was due to de-novo influx, rather than by local changes in cellular localization. To test this hypothesis, we identified the structural architecture of the corpus luteum by immunohistochemistry and compared the localization of
macrophages throughout the luteal phase and after luteal 'rescue' with exogenous HCG. In the final part of the study, we investigated the site of action of HCG on macrophages during luteal 'rescue' by localizing luteinizing hormone (LH)/HCG receptors in the corpus luteum by isotopic in-situ hybridization.

Materials and methods

Source of reagents
All reagents were obtained from Sigma Chemical (Poole, Dorset, UK), unless otherwise stated. The mouse monoclonal antibodies to human CD68 (PG-M1) and von Willebrand factor were obtained commercially (Dako Ltd, High Wycombe, Bucks, UK). The polyclonal rabbit antibody to human 17α-hydroxylase was kindly provided by Prof. M.R. Waterman (Vanderbilt University, Nashville, TN, USA). The polyclonal rabbit antibody to human placental type I 3β-hydroxysteroid dehydrogenase (3β-HSD) was kindly provided by Prof. Van Lau-The (CHUL Research Centre, Quebec, Canada). A 1.5-kb cDNA construct, corresponding to nucleotide 542 to the last nucleotide of the open reading frame (2124), of the human LH receptor in pBluescript (Stratagene, Cambridge, Cambs, UK) was kindly provided by Dr M. Ager of the Faculté de Médecine de Bicêtre, Université Paris-Sud, Le Kremlin-Bicêtre, France.

Collection of tissue
Corpora lutea were enucleated at the time of hysterectomy in 16 women undergoing surgery for benign conditions, typically dysmenorrhea, uterine fibroids or menorrhagia. All women were healthy, aged 32–45 years with regular menstrual cycles and had not received any form of hormonal treatment for at least 3 months prior to taking part in the study. The corpora lutea were dated on the basis of serial urinary LH measurements on samples collected daily prior to operation (Djahanbakhch et al., 1981a). On this basis, four corpora lutea were classified as early-LH(1 to LH>5), four as mid- (LH>6 to LH>10) and four as late- (LH>11 to LH>14) luteal. In addition, four women received daily intramuscular injections of HCG (Profasi; Serono Laboratories, Welwyn Garden City, UK) from LH+7 in daily doubling doses, starting at 125 IU, for 5 to 8 days until surgery. This regimen has been shown to reproduce the hormonal changes of early pregnancy (Illingworth et al., 1990).

The whole corpus luteum was enucleated from the ovary by blunt dissection and the ovary overseen as previously described (Duncan et al., 1996a,b). The tissue was divided immediately into radial blocks in order to ensure that the whole thickness of the gland was represented in any piece. One piece was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin wax for subsequent immunohistochemistry and another piece was frozen in embedding medium (Tissue-Tek OCT compound, Miles Inc., Elkhart, IN, USA) and stored at −70°C. Frozen sections (5 µm) were cut from this block on to poly-L-lysine (50 µg/ml)-coated slides and stored at −70°C until use. In each case an endometrial biopsy was also fixed in paraformaldehyde and processed into paraffin wax for luteal-phase dating by tissue morphometry (Li et al., 1988). Plasma was taken before surgery and progesterone concentration was measured by a standard radioimmunoassay (Djahanbakhch et al., 1981b). This study was approved by the Reproductive Medicine Subcommittee of the South East Scotland Research Ethics Committee, and informed consent was obtained from all patients prior to tissue collection.

Immunohistochemistry
Five-micrometre paraffin wax sections were cut on to poly-L-lysine (50 µg/ml)-coated slides, de-waxed and rehydrated. As preliminary experiments indicated that antigen retrieval using trypsinization was necessary for the detection of the CD68 antigen and von Willebrand factor, these sections were incubated in 0.1% (w/v) trypsin with 0.1% (w/v) calcium chloride, buffered to pH 7.4 with 0.25 M Tris–HCl, for 30 min at 37°C. The sections were then washed in 0.05 M Tris-buffered saline (TBS) pH 8. Endogenous peroxidase activity was blocked with 2% (v/v) hydrogen peroxide in 60% methanol for 30 min at room temperature. This tissue was then permeabilized with 0.1% Triton-X100 in TBS and rinsed in TBS prior to blocking with 20% (v/v) normal rabbit serum in TBS with 4% (w/v) bovine serum albumin for 20 min. Sections were incubated with the primary antibody, monoclonal mouse anti-human macrophage CD68 antigen diluted 1:50 in TBS, or the mouse anti-human von Willebrand factor diluted 1:25, for 1 h at room temperature (Rodger et al., 1997). Mouse immunoglobulin G (IgG) (Vector Laboratories, Peterborough, Cambs, UK) at an equivalent antibody concentration was used as a negative control.

Antibody binding was indicated by an avidin–biotin horseradish peroxidase (Dako Ltd) reaction with a biotinylated rabbit anti-mouse (Dako Ltd) secondary antibody at a dilution of 1:100 in TBS. The reaction was developed with diaminobenzidine to give a stable brown end-product (Vector Laboratories). Sections were then washed in water, counter-stained with haematoxylin, dehydrated through graded alcohols and mounted with Pertex mounting medium (Cellpath, Hemel Hempstead, Herts, UK).

Steroidogenic cells were identified in serial sections by immunohistochemistry for 3β-HSD (Riley et al., 1992). Here, polyclonal rabbit anti-human 3β-HSD was used in a dilution of 1:1000. Immunohistochemistry was performed as described above, without trypsinization, using normal goat serum (SAPU, Carluke, Lanarkshire, UK) to block non-specific binding, and specific binding was detected using biotinylated goat anti-rabbit immunoglobulins (Dako Ltd). Steroidogenic cells of thecae origin were identified in serial sections by immunohistochemistry for 17α-hydroxylase as described previously (Rodger et al., 1995; Duncan et al., 1996a). Briefly, the polyclonal rabbit anti-human 17α-hydroxylase antibody was used at a 1:750 dilution, normal goat serum was used to block non-specific binding, and biotinylated goat anti-rabbit immunoglobulins were used as the secondary antibody. Rabbit serum with an equivalent immunoglobulin concentration (Dako Ltd) was used as a negative control.

In-situ hybridization
Isotopic in-situ hybridization for LH/HCG receptors was performed using antisense and sense [35S]-labelled riboprobes as described previously (Duncan et al., 1996b). Briefly, the antisense probe, incorporating [35S]-labelled UTP (Amersham International plc, Aylesbury, Bucks, UK), was generated from the plasmid vector linearized by HindIII (Promega, Southampton, Hants, UK) to block non-specific binding, and specific binding was detected using biotinylated goat anti-rabbit immunoglobulins (Dako Ltd). Steroidogenic cells of thecae origin were identified in serial sections by immunohistochemistry for 17α-hydroxylase as described previously (Rodger et al., 1995; Duncan et al., 1996a). Briefly, the polyclonal rabbit anti-human 17α-hydroxylase antibody was used at a 1:750 dilution, normal goat serum was used to block non-specific binding, and biotinylated goat anti-rabbit immunoglobulins were used as the secondary antibody. Rabbit serum with an equivalent immunoglobulin concentration (Dako Ltd) was used as a negative control.

As preliminary experiments with fixed serial sections gave technically poor results, LH/HCG receptor mRNA was localized in frozen sections from the same corpora lutea. Frozen sections (5 µm) were fixed in 4% paraformaldehyde, rinsed and then acetylated in 0.25% (v/v) acetic anhydride (BDH Laboratory Supplies, Poole, Dorset, UK). After dehydrating through graded alcohols and drying under vacuum, 100 µl of hybridization buffer (50% deionized formamide, 10% dextran sulphate, 1× Denhardt’s solution, 0.5 mg/ml yeast tRNA, 10 mM dithiothreitol, 0.3 M NaCl, 10 mM Tris, 1 mM EDTA, pH 8) containing 1×106 cpm radiolabelled riboprobe was added to each section, and the slides were incubated overnight at 55°C in a moist chamber.
The following day the slides were treated with RNase A (20 µg/ml), and washed in increasingly stringent conditions. The sections were then dehydrated through graded alcohols, allowed to dry and dipped in Kodak NTB-2 photographic emulsion (IBI Ltd, Cambridge, Cambs, UK). After incubation in the dark for 21 days, they were developed (Kodak D-19) and fixed (Kodak Unifix) at 15°C. The sections were then rinsed, counter-stained with haematoxylin and mounted. They were viewed under dark-field illumination, and the localization of the silver grains was determined by reference to the image viewed under light-field illumination.

**Image analysis**

The number of macrophages was counted by an observer blinded to the tissue identity, and this was repeated the following week to confirm the reproducibility of the results. Macrophage numbers in all sections during the repeat count were within 5% of the initial count. Macrophages were identified by intense brown staining on tissue sections. Only positive cells where the nuclei could be identified were counted in order to avoid counting tiny fragments of cells present in the tissue section. Sections were analysed using a stratified random sampling technique using a graticule lens. The stratified random sampling technique involved taking random fields from a grid of 24 fields arranged around a fixed, non-random, point (the centre of the section). At least five fields of each section were counted and the running mean was monitored to confirm adequate sampling. The granulosa–lutein cell layers, the theca–lutein cell layers and the surrounding stroma were identified by morphology and by comparison with serial sections immunostained for 3β-HSD and 17α-hydroxylase. In addition to the total number of macrophages, the number of macrophages in these layers was also recorded.

The number of macrophages at different stages of the luteal phase, and the number in the different cellular layers, were analysed by one-way analysis of variance with a 5% level of significance. Where significant differences were found to exist, pairwise comparisons using the Bonferroni/Dunn method were performed using commercial computer software (StatView 4.0; Abacus Concepts Inc., Berkeley, CA, USA).

**Results**

**Plasma progesterone concentrations**

The classification of the corpora lutea by serial urinary LH measurement agreed with the luteal-phase dating of endometrial biopsies using the method of Li et al. (1988). As reported previously (Duncan et al., 1996b), the plasma progesterone concentrations were 36.36 ± 9.28 nmol/l in the early luteal samples, 40.35 ± 9.88 nmol/l in the mid-luteal samples and 18.80 ± 12.81 nmol/l in the late luteal samples. After luteal ‘rescue’ by exogenous HCG the plasma progesterone concentrations had increased to 52.75 ± 1.09 nmol/l.

**Functional anatomy of the corpus luteum**

Steroidogenic cells were identified in human corpora lutea by the immunolocalization of 3β-HSD (Figure 1a). Luteal cells of both theca and granulosal origin express this enzyme. The theca–lutein cells were specifically identified by immunolocalization of 17α-hydroxylase (Figure 1b). These cells formed clearly distinct populations, located around the peripheral margin of the granulosa–lutein cells. Vascular endothelial cells were localized by immunohistochemistry for von Willebrand factor (Figure 1c). The theca–lutein cell layer had a rich blood supply. Endothelial cells were also scattered throughout the granulosa–lutein cell layers, particularly in the radial invaginations from the theca–lutein cell layer (Figure 1d). LH receptors were localized by isotopic mRNA in-situ hybridization (Figure 1e). As described previously, they were localized to the steroidogenic cell population (Duncan et al., 1996b). When compared with immunostained sections, no hybridization signal could be detected in endothelial cells, stromal cells or cells lacking the morphological characteristics of steroidogenic cells.

**Localization of tissue macrophages**

Macrophages, as described by immunohistochemical localization of the CD68 antigen, could be localized in all corpora lutea studied (Figure 2a). No staining was present in negative control sections where the primary antibody was replaced with an equivalent concentration of IgG (Figure 2b). Many more macrophages could be detected in late-luteal corpora lutea (Figure 2c) than after luteal ‘rescue’ with exogenous HCG (Figure 2d). The

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**Figure 1.** Functional anatomy of the human corpus luteum: (a) mid-luteal corpus luteum immunostained for 3β-hydroxysteroid dehydrogenase showing staining of the steroidogenic cells with no staining of the connective tissue core; (b) the same corpus luteum as (a) immunostained for 17α-hydroxylase showing staining in the theca–lutein cells; (c) the same corpus luteum as (a) immunostained for von Willebrand factor to demonstrate the endothelial cells; (d) a closer view of the granulosa–lutein cell layer of another mid-luteal corpus luteum immunostained for von Willebrand factor, showing strong endothelial cell immunostaining in the vascular connective tissue invaginations (arrow); (e) dark-field view of a mid-luteal corpus luteum after isotopic in-situ hybridization for luteinizing hormone (LH) receptor mRNA, showing grains distributed over the steroidogenic cells; and (f) the same corpus luteum as (e) after immunohistochemistry for macrophages (CD68 positive cells) showing the distribution of macrophages around the periphery of the granulosa–lutein cell layer, a different localization from LH receptor mRNA. (G) Granulosa–lutein cell layer, (T) Theca–lutein cell layer (arrow) in (a) to (d) connective tissue invaginations. Scale bar = 100 µm.
numbers of macrophages in the corpora lutea at different stages of the luteal phase were counted. The macrophage content of the corpus luteum increased throughout the luteal phase reaching a maximum in the late-luteal phase ($P < 0.05$) (Figure 3). Luteal ‘rescue’ with HCG was associated with a reduction in the number of macrophages (Figure 3), which was significantly lower than in the late-luteal phase ($P < 0.001$).

Macrophages were particularly prominent in the theca–lutein cell layer at all stages (Figure 1f, Figure 2a). In the granulosa–lutein cell layer, they were usually seen in association with the vascular in-foldings, particularly in the late-luteal phase (Figure 2c). The numbers of macrophages specifically located within the granulosa cell layer showed the same pattern as the overall macrophage content throughout the luteal phase (Figure 4). There were no differences in the percentages of macrophages specifically located to the granulosa–lutein cell layer at any stage of the luteal phase. The localization of the LH receptor (Figure 1e) in the human corpus luteum was clearly different from the localization of CD-68 positive tissue macrophages (Figure 1f).

**Discussion**

We have compared the macrophage content in the corpora lutea of women who received exogenous HCG at concentrations equivalent to that of early pregnancy with that of corpora lutea obtained from untreated women at clearly defined stages of the luteal phase. We found that the macrophage content was significantly lower in the HCG-treated women than in the untreated women from the same stage of the luteal phase. This suggests that macrophage accumulation is associated with the loss of luteal integrity during luteolysis and that one of the
Macrophages and luteal ‘rescue’

Effects of HCG during luteal ‘rescue’ is to prevent the accumulation of macrophages in the corpus luteum.

In this study we have concentrated on the numbers and localization of macrophages in the corpus luteum. Various other immune cells have also been identified in the corpus luteum, including polymorphonuclear leukocytes (Brännström et al., 1997), pigs (Hehnke et al., 1992; Wang et al., 1992b), reactive oxygen species (Vega et al., 1995; Kato et al., 1997) and interleukins (Sjögren et al., 1991) have all been shown to inhibit the steroidogenic pathway. In addition, cell death by apoptosis is a feature of luteolysis in many species (Dharmarajan et al., 1994; Zheng et al., 1994; Shikone et al., 1996) and apoptosis can be promoted by macrophage products, such as free oxygen radicals, TNFα and some interleukins (Hale et al., 1996; Jacobson, 1996; Spencer et al., 1996). Macrophages may have a role in structural luteolysis; they can clear cellular debris by phagocytosis (Paavola, 1979) and activate the matrix metalloproteinase enzymes (Hurwitz et al., 1993; Hulbey et al., 1997) which have been implicated in the remodelling associated with luteolysis (Endo et al., 1993; Luck and Zhao, 1995). Whether it is a cause or consequence of falling progesterone synthesis, it is likely that the accumulation of macrophages in the late-luteal phase has a negative effect on the structure and function of the corpus luteum.

Macrophages, however, have been shown to have both pro- and luteotrophic properties under some conditions. It remains possible that the macrophage accumulation in the functional luteal phase is a luteotrophic response to falling progesterone synthesis. Macrophage-derived products have been shown to enhance progesterone output from luteal cells in culture (Kirsch et al., 1983; Halme et al., 1985), and macrophages may secrete factors important for angiogenesis and tissue reorganization. Brännström and Norman (1993) postulated a luteotrophic effect of macrophages in the early luteal phase. In addition, as macrophages could enhance progesterone synthesis (Kirsch et al., 1981, 1983) and promote proliferation of granulosa cells (Fukumatsu et al., 1992), Bukovsky et al. (1995) also proposed a luteotropic role of macrophages. In the rat corpus luteum, non-steroidogenic cells, probably white blood cells, have also been shown to have potent stimulatory effects on luteal cell steroidogenesis (Nelson et al., 1992). It is not clear, therefore, whether the accumulation of macrophages in the corpus luteum would always be associated with a fall in progesterone synthesis.

We have demonstrated that luteal ‘rescue’ with exogenous HCG to mimic the early stages of pregnancy is associated with a reduction in the numbers of macrophages in the late-luteal corpus luteum. This supports the hypothesis that macrophages have a primarily luteolytic, rather than luteotrophic, role in the human corpus luteum. Our findings are different from those of some sub-primate species. Brännström et al. (1994b) failed to find an increase in luteal macrophages in the late-luteal phase. In contrast, other studies (Lei et al., 1991; Best et al., 1996) reported increased macrophages in the functional corpora lutea in the late-luteal phase. It is likely that the discrepant findings of Brännström et al. (1994b) are explained by their more extended definition of the late-luteal phase (from LH+8) and the fact that macrophage influx is a feature of luteal ageing.

The role of macrophage accumulation in the late-luteal phase is not fully established. It is not clear whether it is a cause or consequence of falling progesterone synthesis. Clearly macrophage products can inhibit steroidogenesis in vitro. Nitric oxide (Van Voorhis et al., 1994), TNFα (Benyo and Pate, 1992; Wang et al., 1992b), PGF2α (Grusenmeyer and Pate, 1992), reactive oxygen species (Vega et al., 1995; Kato et al., 1997) and interleukins (Sjögren et al., 1991) have all been shown to inhibit the steroidogenic pathway. In addition, cell death by apoptosis is a feature of luteolysis in many species (Dharmarajan et al., 1994; Zheng et al., 1994; Shikone et al., 1996) and apoptosis can be promoted by macrophage products, such as free oxygen radicals, TNFα and some interleukins (Hale et al., 1996; Jacobson, 1996; Spencer et al., 1996). Macrophages may have a role in structural luteolysis; they can clear cellular debris by phagocytosis (Paavola, 1979) and activate the matrix metalloproteinase enzymes (Hurwitz et al., 1993; Hulbey et al., 1997) which have been implicated in the remodelling associated with luteolysis (Endo et al., 1993; Luck and Zhao, 1995). Whether it is a cause or consequence of falling progesterone synthesis, it is likely that the accumulation of macrophages in the late-luteal phase has a negative effect on the structure and function of the corpus luteum.

### Figure 4. Macrophage numbers within the granulosa–lutein cell layer: numbers of CD68 positive cells in sections of human corpus luteum in the early-LH+1 to LH+5, mid- (LH+6 to LH+10) and late- (LH+11 to LH+14) luteal phase and after luteal ‘rescue’ with human chorionic gonadotrophin (HCG) (HCG×5 to HCG×8).

Values are mean ± SEM (n = 4 per group). Values were analysed by one-way analysis of variance and, where significant differences at the 5% level were observed, pairwise comparisons were conducted using the Bonferroni/Dunn method (*P < 0.05).
et al. (1994a) found particularly high concentrations of macrophages in the rat corpus luteum during the early stages of pregnancy and pseudopregnancy. They found sixfold more macrophages in the corpus luteum of early pregnancy than during luteolysis (Brännström et al., 1994a). This accumulation of macrophages is also seen in the corpus luteum of pregnant rabbits (Bagavandoss et al., 1990). In the rabbit corpus luteum, oestrogen withdrawal induces macrophage invasion, but subsequent oestrogen replacement maintained progesterone production and did not necessarily reduce macrophage numbers (Naftalin et al., 1997). Indeed, in that model system, the relative numbers of macrophages had no apparent relationship to progesterone synthesis. They concluded that the presence of macrophages did not preclude the continuation of progesterone production (Naftalin et al., 1997). It is not known whether the human corpus luteum can continue to function in the presence of increasing numbers of macrophages but, clearly, macrophage influx is not a feature of luteal ‘rescue’ with HCG.

What promotes the influx of macrophages into the failing corpus luteum? Several chemotactic and macrophage-stimulatory molecules have now been identified, including cytokines such as interleukin 8 (Norman and Brännström, 1994), granulocyte–macrophage colony stimulating factor (Nicola, 1989) and monocyte chemoattractant protein 1 (MCP-1) (Leonard and Yoshimura, 1990). These molecules can be detected in the ovary (Robertson and Seamark, 1990; Zhao et al., 1995; Arici et al., 1997). Recently, Townson et al. (1996) reported increased expression of MCP-1 in the corpus luteum during luteal regression in rats. This increase preceded the appearance of macrophages in the corpus luteum and they concluded that MCP-1 may have a prominent role in the immunological process of luteal regression. Induction of structural luteolysis by progesterone receptors in rat corpus luteum (Bowen et al., 1996), and by oestrogen withdrawal in the rabbit corpus luteum (Naftalin et al., 1997), is associated with macrophage accumulation and expression of MCP-1. MCP-1 can be stimulated by cytokines (Oppenheim et al., 1991; Arici et al., 1997) which can be found in the corpus luteum during luteolysis (Brännström and Norman, 1993). However, MCP-1 expression can also be stimulated by HCG in granulosa–lutein cell culture (Arici et al., 1997). Data on the expression of these chemotactic molecules in the human corpus luteum throughout the luteal phase are not yet available, but would be of great interest.

How does exposure to HCG during luteal ‘rescue’ prevent the influx of macrophages into the corpus luteum? HCG exerts its biological actions by binding to, and activating, LH receptors (Cole et al., 1973). LH receptors are localized to the steroidogenic cells of the corpus luteum (Nishimori et al., 1995; Duncan et al., 1996b), and we have shown the localization of these receptors is different from the localization of macrophages. Although we were not able to co-localize macrophages and LH receptors on the same tissue section, it is unlikely that macrophages themselves express the LH receptor. The effect of HCG on macrophage accumulation therefore seems to be mediated through the steroidogenic cells. This effect is likely to be associated with the production or withdrawal of steroid or non-steroid molecules from the cells expressing LH/HCG receptors. Progesterone itself may function as a signal molecule as progesterone receptors can be localized to the human corpus luteum (Suzuki et al., 1994). While these receptors appear to be present on steroidogenic cells (Suzuki et al., 1994; Hild-Petito and Fazleabas, 1997), they can also be found on other cells within the connective tissue stroma (Suzuki et al., 1994). It is not known whether luteal macrophages express progesterone receptors or whether luteal sex steroids can affect migration directly.

In our study, macrophages were found to be associated with the theca–lutein cell layer and the vasculature of the corpus luteum. An early study reported that macrophages were predominant in the granulosa–lutein cell layer of the human corpus luteum (Gillim et al., 1969). However, later studies found a predominance in the theca–lutein layer (Wang et al., 1992a, Brännström et al., 1994b). We have used steroidogenic markers to confirm this observation. Brännström et al. (1994b) reported that macrophages were more abundant in the theca–lutein layer and were particularly associated with blood vessels. The relationship with blood vessels may suggest a recruitment of monocytes/macrophages from the circulation. In our study macrophage numbers increased during the late-luteal phase, in all cellular compartments. This is consistent with recruitment from the circulation rather than local migration. Interestingly, MCP-1 is secreted by several cell types including endothelial cells and fibroblasts (Leonard and Yoshimura, 1990). In the rat corpus luteum, luteal vascular cells appear to be a source of MCP-1 (Townson et al., 1996). The endothelial cells and their communication with the steroidogenic cells of the corpus luteum may play a major role in the control of macrophage recruitment.

In conclusion, this study has shown that macrophages accumulate in the human corpus luteum during the luteal phase and are maximal in the late-luteal phase. One of the effects of HCG during luteal ‘rescue’ is to prevent this influx of macrophages into the corpus luteum. As macrophages do not express LH/HCG receptors, this effect is mediated indirectly through factors produced by the steroidogenic cells. Further work is needed to study the expression of chemotactic molecules in the human corpus luteum throughout the luteal phase and after luteal ‘rescue’ with HCG.

Acknowledgements
We acknowledge Dr H.M.Fraser, Dr S.F.Lunn, Mr M.R.Millar, Dr R.M.Shiels and Professor A.S.McNeilly for helpful discussion during the course of this project. We are indebted to Dr M.Atger for providing the LH receptor cDNA probe, Professor M.R.Waterman and Professor Van Luu-The for providing antibodies and Dr G.F.Erickson for providing a copy of his protocol for in-situ hybridization. Mrs V.Reid-Thomas helped in the identification and recruitment of patients. Dr P.E.Rodger is a clinical research fellow supported by the Medical Research Council and Dr W.C.Duncan is a clinical research fellow supported by the Wellcome Trust.

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Received on February 13, 1998; accepted on June 10, 1998