Immune cells in the corpus luteum: friends or foes?

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The corpus luteum produces progesterone, which is essential for the maintenance of pregnancy. In the absence of a viable embryo, the corpus luteum must regress rapidly to allow for development of new ovulatory follicles. In many species, luteal regression is initiated by uterine release of $\text{PGF}_{2\alpha}$, which inhibits steroidogenesis and may launch a cascade of events leading to the ultimate demise of the tissue. Immune cells, primarily macrophages and T lymphocytes, are present in the corpus luteum, particularly at the time of luteolysis. The macrophages are important for ingestion of cellular remnants that result from the death of luteal cells. However, it has also been hypothesized that immune cells are involved directly in the destruction of luteal cells, as well as in the loss of steroidogenesis; this hypothesis is reviewed in the first part of this article. An alternative hypothesis is also presented, namely that immune cells serve to abate an inflammatory response generated by dead and dying luteal cells, in effect, preventing a response that would otherwise damage surrounding ovarian tissues. Finally, the changes in immune cells that accompany maternal recognition of pregnancy and rescue of the corpus luteum are discussed briefly. Inhibition of immune cells in the corpus luteum during early pregnancy may be due to embryonic or uterine signals, or to maintenance of high progesterone concentrations within the luteal tissue.

The primary hormonal regulators of the corpus luteum have been studied in depth. Progesterone synthesis is driven by luteotrophic hormones, most commonly LH, although in some species prolactin, FSH or even oestradiol serve as luteotrophic hormones. Much has been learned about the intracellular signal transduction cascades activated by the luteotrophic hormones and the mechanisms involved in regulation of steroidogenic capacity. The corpus luteum is a transitory endocrine gland; thus, in the absence of the appropriate embryonic signal, luteolysis will ensue. In most species other than primates, luteal regression is initiated by the release of $\text{PGF}_{2\alpha}$ from the uterus, which causes a cascade of events within the corpus luteum that ultimately leads to demise of the tissue (Fig. 1). The primary functional effect of $\text{PGF}_{2\alpha}$ is to inhibit steroidogenesis stimulated by LH and both high density (HDL) and low density lipoprotein (LDL). The action of $\text{PGF}_{2\alpha}$ on luteal cells is transduced via the phospholipase C (PLC), protein kinase C (PKC) pathway, and the immediate effect of $\text{PGF}_{2\alpha}$ is to prevent transport of cholesterol through the mitochondrial membrane. This effect probably occurs as a result of inhibition of synthesis or activity of the steroidogenic acute regulatory protein (StAR), and perhaps other proteins that are important for cholesterol transport. Prostaglandin synthase is stimulated by $\text{PGF}_{2\alpha}$, allowing for increased synthesis of luteal prostaglandins. Endothelial cells release endothelin 1 in

response to $\text{PGF}_{2\alpha}$, which inhibits steroidogenesis by unknown mechanisms. Receptors for $\text{PGF}_{2\alpha}$ have been identified on large steroidogenic luteal cells. If there are no prostaglandin receptors on small luteal cells, there must be an as yet unidentified factor that communicates the luteolytic signal from large to small steroidogenic cells. After the initial decrease in progesterone, more chronic effects of $\text{PGF}_{2\alpha}$ include loss of gonadotrophin receptors and disruption of the cytoskeleton; the latter may prevent movement of steroidogenic carrier protein 2-coupled cholesterol through the cytoplasm, or secretion of progesterone. An additional chronic effect of $\text{PGF}_{2\alpha}$ in association with endothelin 1, is to cause vasoconstriction of luteal capillaries and apoptosis of capillary endothelial cells, restricting access of steroidogenic cells to gonadotrophin and oxygen. This effect is followed by morphological changes in steroidogenic cells indicative of apoptosis, and a loss in both size and number of first large and then small steroidogenic luteal cells. The model presented (Fig. 1) is a result of the work of numerous investigators, and has been reviewed thoroughly by Niswender *et al.* (2000). Accompanying the changes depicted is the appearance of immune cells as the corpus luteum regresses. The physiological role of these cells has been the subject of debate, which is addressed in this review.

There may be distinct mechanisms to achieve the loss of progesterone and the structural degeneration of the tissue. This is evidenced by the fact that withdrawal of

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Fig. 1. Prostaglandin (PG)-induced cascade of events that contribute to luteal regression. Intracellular events that occur include activation of phospholipase C (PLC), the protein kinase C (PKC) system, and prostaglandin synthesis via prostaglandin endoperoxide synthase (PGS). Cholesterol translocation to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR) is inhibited, resulting in an acute reduction in progesterone synthesis (P_4). In addition, endothelial cells in the corpus luteum are targets of $PGF_{2\alpha}$, releasing endothelin 1 (ET-1) which acts on luteal cells via the ET-A receptor, inhibiting progesterone synthesis. Endothelin 1 and $PGF_{2\alpha}$ are also potent vasoconstrictive agents, the actions of which cause a reduction in the luteal blood supply, initiating apoptotic mechanisms that facilitate luteal regression further. Although there is substantial evidence demonstrating communication between small (SLC) and large (LLC) luteal cells, the mechanisms by which luteolytic signals may be conveyed from large to small luteal cells remains unknown. Cyt P_{450} : cytochrome P_{450} side-chain cleavage enzyme; HDL: high density lipoprotein; LDL: low density lipoprotein; SCP_2 : sterol carrier protein 2.

gonadotrophic support leads to a decrease in progesterone synthesis, yet the corpus luteum retains the capacity for steroidogenesis (Hutchinson and Zeleznik, 1985), and the effects of $PGF_{2\alpha}$ *in vivo* are reversed when cells are placed *in vitro* (Pate and Nephew, 1988). The decline in progesterone production *in vivo* is a hallmark of luteolysis, yet it is not sufficient to bring about structural regression of the corpus luteum (Jenkin *et al.*, 1984), and $PGF_{2\alpha}$ *in vitro* can inhibit LH-stimulated steroidogenesis without altering cell viability (Pate and Condon, 1984). Therefore, although $PGF_{2\alpha}$ may initiate the loss in steroidogenesis, additional mechanisms must come into play for luteolysis to be completed. These mechanisms, which involve the participation of immune cells, may represent a feature common to luteolysis in all species, including those that are not dependent on uterine prostaglandin to initiate regression.

The role of immune cells in the corpus luteum can be described using two hypotheses. The first hypothesis, which now is supported by considerable evidence, is that immune cells are involved actively in the loss of steroidogenesis and

destruction of the tissue. The second hypothesis is that immune cells control a potential inflammatory condition caused by dying cells, and provide a nurturing environment as the cells of the corpus luteum die progressively. Finally, the inhibition of immune cells in the corpus luteum during maternal recognition of pregnancy is discussed briefly. It is not the purpose of this review to provide a comprehensive overview of luteolysis (see Fig. 1 and Niswender *et al.*, 2000). Rather, the intention is to stimulate the reader to think of alternative hypotheses that may explain how immune cells participate in the regression of the corpus luteum.

Immune cells in the corpus luteum

Lobel and Levy (1968) first described the presence of white blood cells in the bovine corpus luteum. They observed that lymphocytes were present in the connective tissue surrounding the luteal vasculature on day 14 of the oestrous cycle. On days 15–17, the lymphocytes infiltrated among the luteal cells, and by day 19 macrophages were also present. The macrophages were later shown to be involved

in phagocytosis of cells and cell remnants (Paavola, 1979). Subsequent studies confirmed that lymphocytes and macrophages are present in the corpus luteum throughout the luteal phase (Kirsch *et al.*, 1981; Hume *et al.*, 1984; Bagavandoss *et al.*, 1988). Although there has been one report that there are more macrophages in luteal tissue during the mid-luteal phase (Gaytán *et al.*, 1998a), most investigators have observed an increase in lymphocytes or macrophages in the corpus luteum at the time of luteolysis (Bagavandoss *et al.*, 1988; Best *et al.*, 1996; Takaya *et al.*, 1997; Penny *et al.*, 1999; Bauer *et al.*, 2001). Withdrawal of the luteotrophic hormone oestradiol from the rabbit corpus luteum results in infiltration of macrophages (Naftalin *et al.*, 1997). In rats, macrophage infiltration is associated with the pro-oestrous prolactin surge (Gaytán *et al.*, 1997; Bowen *et al.*, 1999) or a luteolytic injection of prolactin in hypophysectomized rats (Bowen *et al.*, 1996). Recruitment of macrophages into the corpus luteum of rats, rabbits and cows is probably regulated by expression of monocyte chemoattractant protein 1 (MCP-1; Bowen *et al.*, 1996, 1999; Townson *et al.*, 1996; Penny *et al.*, 1998; Penny, 2000). In each of these species there appears to be an increase in expression of MCP-1 at the time of luteolysis, and this is associated with the influx of macrophages. Olson and Townson (2000) have recently reported that intercellular adhesion molecule 1 (ICAM-1) is also expressed during prolactin-induced luteal regression in rats and its expression is associated with the recruitment of monocytes and macrophages into the corpus luteum. Therefore, there are at least two molecules that are increased at the time of luteal regression that may serve to attract, activate and promote adhesion of immune cells in the tissue. Little is known about the molecules involved in recruitment or activation of lymphocytes in luteal tissue. Bauer *et al.* (2001) reported that most of the T lymphocytes in the corpus luteum migrate into the tissue, but that there is some proliferation of lymphocytes within the tissue. The presence and increase of lymphocytes at the time of luteolysis implies that they may also function in a manner that facilitates regression. It has been hypothesized that immune cells are involved in the onset and progression of luteolysis and have a role beyond that of merely cleaning up cellular debris (Bukovsky and Presl, 1979; Murdoch, 1987; Bagavandoss *et al.*, 1988; Fairchild and Pate, 1989).

Hypothesis I – immune cells as instruments of destruction: direct participation in loss of steroidogenesis and in the demise of luteal cells and tissue

Hormonal regulation of luteolysis and the role of immune cells in the loss of steroidogenesis

Non-steroidogenic cells out-number steroidogenic cells in the corpus luteum, and probably participate in the regulation of steroidogenic cell function. Milvae (2000) reviewed the relationships between the endothelial cells

and steroidogenic cells, with particular emphasis on the hypothesized role of endothelin 1. Endothelial cells, fibroblasts and immune cells secrete a variety of potential paracrine regulators of ovarian function, such as growth factors, prostaglandins and lipoxygenase products. As macrophages and T lymphocytes become activated within a tissue they secrete cytokines, which potentiate the immune response. Cytokines may exert either positive or negative effects on endocrine cell function. Both the synthesis and action of oestradiol, which is a luteotrophic hormone in pigs, are inhibited by tumour necrosis factor α (TNF- α ; Wuttke *et al.*, 1997a). In microdialysed pig corpus luteum, PGF_{2 α} causes an increase in progesterone production. However, when corpora lutea are pretreated with TNF- α , PGF_{2 α} brings about a further reduction in progesterone over that caused by TNF- α alone (Wuttke *et al.*, 1998). These authors speculated that TNF- α serves as a signal within the pig corpus luteum that switches the action of PGF_{2 α} from luteotrophic to luteolytic. It is plausible that the luteolytic action of PGF_{2 α} is mediated by local release of a number of cytokines that may cause a further decrease in progesterone production. Cytokines may mediate PGF_{2 α} -induced increases in phospholipase A₂, formation of reactive oxygen species and apoptosis, all which occur during luteolysis (Wu and Carlson, 1990; Behrman *et al.*, 1991; Juengel *et al.*, 1993). There may even be a positive feedback between production of cytokines and further release of PGF_{2 α} , as TNF- α can stimulate PGF_{2 α} release from endometrial slices late in the oestrous cycle to a greater extent than can oxytocin (Miyamoto *et al.*, 2000).

TNF- α has been detected in rabbit (Bagavandoss *et al.*, 1990), bovine (Roby and Terranova, 1989), ovine (Ji *et al.*, 1991) and pig (Zhao *et al.*, 1998) corpora lutea, localized primarily to the resident macrophages. However, there are reports that TNF- α is also found in large luteal cells or granulosa cells (Roby and Terranova, 1989; Roby *et al.*, 1990; Zolti *et al.*, 1990; Kondo *et al.*, 1995; Wuttke *et al.*, 1997b). The mRNA for TNF- α is present in the bovine corpus luteum throughout its lifespan (Petroff *et al.*, 1999), but secreted TNF- α protein or bioactivity could be detected only after the initial decrease in plasma progesterone in bovine and ovine corpora lutea (Ji *et al.*, 1991; Shaw and Britt, 1995). These data are consistent with a role for TNF- α in facilitating luteolysis after PGF_{2 α} causes the initial decline in progesterone.

Acute exposure to TNF- α causes a transient increase in luteal progesterone production, whereas chronic exposure results in inhibition of progesterone synthesis by bovine and pig luteal cells (Benyo and Pate, 1992; Pitzel *et al.*, 1993; Townson and Pate, 1996; Wuttke *et al.*, 1998). The anti-steroidogenic effect of TNF- α is due, at least in part, to loss of StAR and LH receptor mRNA (Chen *et al.*, 1999). Receptors for TNF- α have been localized on both small and large luteal cells, but the small cells possess a higher affinity receptor (Richards and Almond, 1994). The inhibitory effect of TNF- α on gonadotrophin-stimulated steroidogenesis is probably mediated via the type I TNF receptor (Roby *et al.*,

1999). In addition to direct effects on steroidogenic cells, some of the effects of TNF- α in the corpus luteum may be exerted on microvascular endothelial cells, which also contain TNF- α receptors (Richards and Almond, 1994; Okuda *et al.*, 1999; Friedman *et al.*, 2000); luteal endothelial cells undergo apoptosis in response to TNF- α (Friedman *et al.*, 2000). Progesterone production by luteal cells is also inhibited by interleukin 1 β (IL-1 β) and interferon γ (IFN- γ ; Nothnick and Pate, 1990; Fairchild and Pate, 1991). These three cytokines are extremely potent stimulators of luteal cell prostaglandin production (Nothnick and Pate, 1990; Fairchild and Pate, 1991; Benyo and Pate, 1992; Townson and Pate, 1996). The anti-steroidogenic effect of the three cytokines is not mediated by the increase in luteal prostaglandin production, because the effect is still apparent in the presence of a concentration of indomethacin that completely blocks the increase in prostaglandin synthesis (Fairchild and Pate, 1991). Thus, these two functional effects of cytokines on luteal cells are not interdependent.

Cytokines as local initiators of cell death

There is evidence that luteal regression occurs via apoptosis (Sawyer *et al.*, 1990; Juengel *et al.*, 1993), although Fraser *et al.* (1999) reported that apoptosis is not involved in luteal regression in marmosets. The factors that regulate the onset of apoptosis in the corpus luteum and the biological basis for the selection of specific cells to undergo apoptosis are not known. Gaytán *et al.* (1998b) demonstrated that prolactin and possibly progesterone are necessary for induction of apoptosis in the rat corpus luteum, but progesterone apparently inhibits apoptosis in the bovine corpus luteum (Rueda *et al.*, 2000). Apoptosis is initiated by TNF- α in a number of types of cell, including luteal cells (Wuttke *et al.*, 1997a; Petroff *et al.*, 1999). In cultured bovine and murine luteal cells, TNF- α alone does not alter cell viability, but exerts a dose-dependent cytotoxic effect in the presence of IFN- γ (Benyo and Pate, 1992; Jo *et al.*, 1995a). This effect is similar to the cytotoxic effect of the combination of these two cytokines that has been demonstrated in pancreatic β islet cells (Campbell *et al.*, 1988; Rabinovitch *et al.*, 1990) and thyroid cells (Weetman and Rees, 1988). As TNF- α and its receptors are present in the corpus luteum, it is possible that this cytokine induces apoptosis of the steroidogenic cells directly, possibly in this way mediating the luteolytic action of PGF_{2 α} .

Reactive oxygen species (ROS) are probably involved in damage of cells during luteal regression, and the generation of ROS may also have negative effects on steroidogenesis. Kato *et al.* (1997) reviewed this subject and noted that, although ROS do not initiate luteal regression, ROS may mediate downstream events in luteal regression that ultimately lead to the demise of the corpus luteum. Production of ROS is stimulated by PGF_{2 α} and activated through the protein kinase C pathway (Sakka *et al.*, 1997; Aten *et al.*, 1998). However, Aten *et al.* (1998) reported that the ROS were generated primarily by non-steroidogenic

cells during luteolysis, and suggested that this was a role of leucocytic cells in promoting rapid damage of the surrounding luteal cells. Progesterone can inhibit superoxide radical production by macrophages (Sugino *et al.*, 1996), possibly protecting luteal cells from cellular damage before the decline in steroidogenesis. The high rate of steroidogenesis characteristic of a fully functional corpus luteum also results in substantial production of ROS. In addition to possible protection by progesterone and by endogenous antioxidants such as ascorbate, α -tocopherol and β -carotene (Rapoport *et al.*, 1998), luteotrophic hormones, such as prolactin and hCG, can increase enzymes that protect against ROS, such as manganese superoxide dismutase (Mn-SOD; Sugino *et al.*, 1998a; Dharmarajan *et al.*, 1999). The decline in Mn-SOD during regression of the corpus luteum (Rueda *et al.*, 1995a) may increase the susceptibility of luteal cells to the ROS generated by activated resident immune cells. Finally, nitric oxide (NO) has also been implicated as a mediator of cell death during luteolysis (Olson *et al.*, 1996). Inhibition of NO synthase results in an increase in progesterone production and extension of the duration of the oestrous cycle in cows (Jaroszewski and Hansel, 2000). IL-1 β -induced cytotoxicity in the rat ovary is mediated by NO (Ellman *et al.*, 1993), and IFN- γ , in combination with either TNF- α or IL-1 β , stimulated NO production in cultured murine luteal cells (Jo *et al.*, 1995b). However, inhibition of NO synthase did not decrease the cytotoxic effect of TNF- α + IFN- γ (Jo *et al.*, 1995b; Petroff *et al.*, 1999).

Apoptosis can be initiated via the Fas–Fas ligand system. Fas is a transmembrane receptor that belongs to the TNF receptor family. When bound by Fas ligand, signal transduction pathways that result in apoptosis are activated. Fas has been implicated as a mediator of cell death during follicular atresia and cytokine-induced death of follicular cells (Quirk *et al.*, 1998; Porter *et al.*, 2000; Vickers *et al.*, 2000). Both Fas and Fas ligand are present in the corpus luteum, and they both increase at the time of luteolysis (Roughton *et al.*, 1999). Prolactin-induced apoptosis in rat luteal cells *in vitro* may be mediated by the Fas–Fas ligand system (Kuranaga *et al.*, 1999). Quirk *et al.* (2000) demonstrated that TNF- α + IFN- γ upregulate Fas mRNA in murine luteal cells, and increase the sensitivity of those cells to killing by Fas monoclonal antibody. Thus, immune cell cytokines may facilitate luteolysis by increasing Fas–Fas ligand-mediated cell killing. Removing immune cells from a suspension of luteal cells prevented prolactin-induced apoptosis (Kuranaga *et al.*, 2000). These authors also found that Fas mRNA was expressed in luteal cells, whereas Fas ligand mRNA was expressed in the T lymphocytes found in the corpus luteum. They concluded that immune cells are required for Fas-mediated apoptosis during luteal regression. Therefore, a primary role of immune cells in the corpus luteum may be to activate the Fas transmembrane protein to initiate cell death. The proposed role of immune cells and cytokines in mediating the decline in steroidogenesis and initiating cell death is depicted (Fig. 2).

Fig. 2. Fully functional luteal cells make progesterone (P_4) in response to stimulation by gonadotrophin, such as LH. High concentrations of progesterone inhibit functions of immune cells and the cytokines they produce. In many species, uterine release of $PGF_{2\alpha}$ initiates luteolysis, and gonadotrophin-stimulated steroidogenesis is acutely inhibited. Resident immune cells may be stimulated either directly or indirectly by $PGF_{2\alpha}$ to secrete cytokines. Tumour necrosis factor α ($TNF-\alpha$) further decreases progesterone production and stimulates synthesis of luteal prostaglandins. The decline in progesterone will be sustained as $TNF-\alpha$ decreases mRNA for steroidogenic acute regulatory protein (StAR) and LH receptor (LH-R). During luteolysis, there is a decline in manganese superoxide dismutase (MnSOD), which increases the susceptibility of luteal cells to reactive oxygen species (ROS). Continued exposure to $TNF-\alpha$ and interferon γ ($IFN-\gamma$) induces expression of class II MHC and Fas molecules. Antigen presentation by MHC molecules activates lymphocytes and further production of cytokines. Recognition of Fas by Fas ligand (FasL) on lymphocytes initiates apoptosis of luteal cells. Activated lymphocytes produce ROS, and lymphocytes that are exposed to interleukin 1β ($IL-1\beta$) may produce nitric oxide (NO), causing further damage to adjacent luteal cells. These events culminate in apoptosis of steroidogenic cells and structural demise of the tissue.

MHC molecules on luteal cells: recognition as non-self

The major histocompatibility complex (MHC) genes encode cell surface glycoproteins that are critical to the recognition of cells by T lymphocytes as either self or non-self. The MHC molecules present small peptides of exogenous or endogenous origin to T lymphocytes. Peptides presented by class I MHC molecules are recognized by CD8⁺ T lymphocytes. If appropriately stimulated, these cells show cytotoxic activity directed against the antigen-presenting cell. Class II MHC molecules and the associated peptides are recognized by CD4⁺ T lymphocytes. Activation of CD4⁺ cells results in clonal expansion of these cells and release of cytokines such as $TNF-\alpha$ and $IFN-\gamma$. Although presentation of peptides by class II molecules is generally restricted to professional antigen-presenting cells, such as macrophages and B cells, there are now numerous examples of somatic cell

expression of class II molecules. This is usually indicative of a pathogenic state, and is a common feature of most autoimmune diseases (Eisenbarth, 1999). Both class I and class II MHC molecules may present self-peptides, thus eliciting an autoimmune response.

Luteal cells express both class I and class II MHC molecules (Fairchild and Pate, 1989; Khoury and Marshall, 1990; Kenny *et al.*, 1991). Exposure of luteal cells to $TNF-\alpha$ or $IFN-\gamma$ increases the expression of class I MHC per cell, as well as the overall percentage of luteal cells expressing class I MHC molecules (Benyo and Pate, 1992). $IFN-\gamma$ treatment also results in a strong induction of class II MHC expression in cultured luteal cells (Fairchild and Pate, 1989). Expression of MHC molecules has also been examined on freshly isolated luteal cells collected from cows on days 6, 10 and 18 of the oestrous cycle, representing developing, fully functional and early regressing corpora lutea, respectively (Benyo *et al.*, 1991).

As expected, class I MHC molecules are present on luteal cells, and their expression does not vary significantly across the oestrous cycle. In contrast, expression of class II MHC molecules is variable, depending on the functional state of the corpus luteum. In the developing corpus luteum, minimum expression of class II molecules is detected, but expression is increased in the mid-cycle corpus luteum. By day 18 of the oestrous cycle (just before luteal regression in cows), most of the small and large luteal cells show substantial class II MHC expression. Expression of class II molecules on luteal cells is also significantly increased when luteal regression is induced by PGF_{2α}. In these studies, MHC-positive luteal cells were identified as steroidogenic cells by their size, as determined by flow cytometry (> 20 μm) and positive staining for the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase. This finding led to the hypothesis that the demise of the corpus luteum may involve local autoimmune response mechanisms facilitated by increased expression of class II MHC molecules at the time of luteolysis (Benyo *et al.*, 1991). Similar patterns of MHC expression have been observed in the sheep corpus luteum (Kenny *et al.*, 1991). In both of these studies, MHC molecule expression was significantly lower in corpora lutea from pregnant animals compared with that in non-pregnant animals, consistent with a role for MHC molecules in luteal regression, and implying that there is a mechanism by which MHC expression in the corpus luteum is either modified or suppressed during maternal recognition of pregnancy.

There is also evidence that these MHC molecules are functional immunologically. Petroff *et al.* (1997) co-cultured luteal cells with autologous T cells and assessed T cell proliferation. It was observed that luteal cells are extremely effective stimulators of T cell proliferation, and the T cell proliferative response was greater in the presence of cells from regressing corpora lutea compared with that of cells from mid-cycle corpora lutea. Furthermore, the degree of T cell proliferation in the presence of luteal cells was 7.5-fold greater than that observed when T cells were stimulated by professional antigen-presenting cells (mixed lymphocyte reaction), indicating that T cell stimulation by luteal cells is not due to minor contamination by resident macrophages. From these observations, it was suggested that luteal cells could serve as antigen-presenting cells, initiating a transient autoimmune response during luteolysis (Petroff *et al.*, 1997). Autoimmunity is an immune response generated against self-antigens, which may occur as part of a physiological event. However, if allowed to continue, the autoimmunity may progress to a disorder, resulting in autoimmune disease, of which there are numerous examples (Bottazzo *et al.*, 1986; Bellgrau and Eisenbarth, 1999). Normal expression of ovarian target antigens may develop into ovarian autoimmune disease (Hill *et al.*, 1990).

The ability of somatic cells in the reproductive tract to act as antigen-presenting cells is not without precedent. Uterine epithelial and stromal cells can serve as antigen-presenting cells (Wira and Rossoll, 1995). These cells show MHC class

II-mediated antigen presentation, which varies with the oestrous cycle and appears to be regulated by steroid hormones (Wira and Rossoll, 1995; Wira *et al.*, 2000). Thus, types of reproductive cell may be active stimulators of resident immune cells to facilitate responses necessary for normal function of the reproductive tissue.

Disruption of the immune response

Additional evidence for a role of immune cells in promoting luteolysis is found in experiments in which the immune system has been disrupted. Immunosuppression by dexamethasone blocked natural, but not PGF_{2α}-induced, luteolysis in rats (Wang *et al.*, 1993) and extended luteal function by an average of 10 days in cows (Kanchev *et al.*, 1976). Experimental suppression of the immune system results in ovarian dysfunction in both rats (Bukovsky *et al.*, 1977) and dairy cows (Alila and Hansel, 1984). Neonatal thymectomy results in severe ovarian dysgenesis, a model often used to study interactions between the immune system and the ovary (Michael *et al.*, 1980), and corpora lutea do not regress normally in athymic nude mice (reviewed by Bukovsky *et al.*, 1991). Possibly the most compelling evidence is that removal of macrophages by splenectomy results in a significant prolongation of luteal function in pseudopregnant rabbits (Nariai *et al.*, 1995; Endo and Kanayama, 1998), the oestrous cycle is prolonged in granulocyte-macrophage colony-stimulating factor (GM-CSF)-deficient mice (Jasper *et al.*, 2000), and dioestrus is extended in TNF type I receptor knockout mice (Roby *et al.*, 1999). Finally, luteal regression after parturition in ewes progresses much less rapidly than at the end of the oestrous cycle, and large luteal cells are still present at day 15 after parturition (O'Shea and Wright, 1985). There must be active mechanisms during cyclic ovarian function to allow rapid deterioration of the corpus luteum, otherwise deterioration would occur more slowly, as is the case after parturition.

Hypothesis II – immune cells protect surviving luteal cells as the corpus luteum regresses

The widely recognized and documented increase in immune cells in the corpus luteum as it regresses has prompted many investigators to invoke these cells and their cytokine products as active participants in the destruction of luteal cells. This hypothesized role for immune cells has a considerable attractiveness, in that the appearance of large numbers of these cells in the regressing corpus luteum might offer a ready explanation for the physical destruction of luteal cells: the immune cells mediate or initiate changes in luteal cells that lead to death of luteal cells primarily by apoptosis. However, the appearance of immune cells at the time of regression can be viewed in another light: namely, that these cells function to control a potential inflammatory condition created by dead and dying cells, to provide a nurturing environment for the cells that have not died, and to participate in continuous restructuring as the corpus luteum loses mass.

In women, the corpus luteum does not regress with a cataclysmic bang, but rather shows a gradual loss of progesterone production over a period of about 1 week (Thornycroft, 1971). During this period of declining progesterone production, immune cells, primarily macrophages and lymphocytes, invade the human corpus luteum (Hameed *et al.*, 1995; Best *et al.*, 1996; Takaya *et al.*, 1997). The corpus luteum is still present in the early follicular phase of the next menstrual cycle, and has abundant macrophages (Brännström *et al.*, 1994). In cows (Kastelic *et al.*, 1990; Peters *et al.*, 1994) and other domestic species (Karsch *et al.*, 1979), in which uterine prostaglandins appear to initiate the cascade of events leading to regression (McCracken *et al.*, 1999; Niswender *et al.*, 2000), the decrease in progesterone in the non-fertile cycle is more precipitous than that in women. However, as the bovine corpus luteum enters the period of declining progesterone production, lymphocytes are more abundant; at advanced stages of regression, macrophages are more abundant than at an earlier stage (Penny *et al.*, 1999; Bauer *et al.*, 2001). Thus, throughout the period during which progesterone secretion is declining, immune cells infiltrate the regressing corpus luteum, composed of dying and living luteal parenchymal cells. The above hypothesis holds that these immune cells are essential to contain a potential inflammatory condition, and possibly to provide local support for the surviving luteal cells until they are induced to die, leaving the corpus albicans.

Steroidogenesis in the presence of immune cells

What changes in steroidogenesis are observed when luteal cells are cultured in the presence of immune cells? Kirsch *et al.* (1981) cultured mouse luteal cells with either peritoneal macrophages or with macrophages derived from corpora lutea, and observed stimulation of progesterone and 20 α -dihydroprogesterone production and accumulation over 3–5 days and 6–8 days of culture. Human granulosa cells in the early stages of luteinization were cultured for 24 or 48 h with either peritoneal macrophages or blood monocytes, and in the presence of these cells, progesterone production and accumulation was increased. Furthermore, the increase in progesterone production and accumulation was positively related to concentrations of peritoneal macrophages (Halme *et al.*, 1985). Naito and Takahashi (1988) cultured rat luteal cells with peritoneal macrophages and observed that, in the presence of macrophages, the secretion of progesterone and the ratio of progesterone:20 α -dihydroprogesterone were maintained well above those of control cultures without macrophages. As macrophages alone did not produce steroids (data not shown), the authors concluded that macrophages can maintain progesterone secretion in luteal cells. Steroidogenesis was not inhibited in rat luteal cells co-cultured with splenic macrophages, and splenic macrophages increased the production of progesterone relative to 20 α -dihydroprogesterone in response to pituitary hormones

(Matsuyama *et al.*, 1992). Castro *et al.* (1998) reported that human luteal cells from which leucocytes were removed produced higher amounts of progesterone in cell cultures, but less progesterone when stimulated with chorionic gonadotrophin. In the cultures, the production of oestradiol was not changed by the absence of leucocytes. Thus, most of the evidence from published studies indicates that macrophages artificially added to luteal cells in culture do not inhibit steroidogenic activity, and actually promote higher rates of steroid production or accumulation, or increase responsiveness to gonadotrophins. An important but unresolved question is whether the presence of macrophages was associated with higher survival rates for luteal cells in these cultures. Some reports have revealed that cytokines normally produced by macrophages and lymphocytes can have salutary effects on luteal or granulosa cells, including increased rates of steroidogenesis (Hughes *et al.*, 1991; Chen *et al.*, 1992; Ness and Kasson, 1995; Prakash *et al.*, 1997) and increased expression of manganese superoxide dismutase that can protect mitochondria against oxygen radicals (Sugino *et al.*, 1998b).

If macrophages and other immune cells function as supportive or protective cells in the dying corpus luteum, luteal tissue infiltrated with macrophages should retain its capacity for progesterone synthesis and secretion. This idea was tested in rabbits by Naftalin *et al.* (1997). In this report, an oestrogen withdrawal–replacement protocol was used to induce, experimentally, invasion of macrophages into the corpora lutea. Corpora lutea of the same age either with experimentally induced large numbers of macrophages or with small numbers of macrophages (control animals without oestradiol withdrawal–replacement) were incubated and progesterone was measured in the medium. Progesterone production and accumulation was maintained throughout 10 h of incubation and was not different; surviving luteal tissue heavily infiltrated with macrophages produced as much progesterone as luteal tissue with relatively few macrophages. A similar observation was made in rats (D. H. Townson, J. M. Bowen, D. G. Remick, J. S. Warren, P. L. Keyes, unpublished). Hypophysectomized rats with corpora lutea were either treated with prolactin to induce an invasion of macrophages into the corpora lutea or were treated with vehicle (controls). After 4 days, when the corpora lutea of prolactin-treated rats were heavily infiltrated with macrophages and were regressing (Bowen *et al.*, 1996), the corpora lutea were removed, incubated for 10 h, and progestin (20 α -dihydroprogesterone plus progesterone) was measured in the conditioned medium. The regressing corpora lutea from prolactin-treated rats produced and accumulated significantly (four- to fivefold; $P < 0.01$) more progestin per unit of wet tissue than did non-regressing corpora lutea of control rats. It is possible that the production of steroid by regressing corpora lutea in these two studies was underestimated as the data were corrected for wet tissue mass, which would include populations of immune cells. Correction based upon the amount of a known steroidogenic enzyme, such as 3 β -hydroxysteroid

dehydrogenase, would be revealing. The above data indicate that the presence of large numbers of macrophages in rabbit and rat luteal tissues is compatible with high rates of steroidogenesis by surviving luteal cells. Furthermore, Amsterdam *et al.* (1998) reported that steroidogenesis may be increased in the early stages of apoptosis. If it is assumed that luteal cells die progressively over a period of days, then a question arises as to the biological basis for selection of luteal cells to undergo apoptosis as the luteal phase progresses and winds down.

Phagocytosis of cells and cell remnants

A major characteristic of macrophages is the capacity for phagocytosis of cells that are damaged or dying (Johnston, 1988). In the corpus luteum, this particular activity may be very important for timely, non-inflammatory resolution of regression. Macrophages engaged in phagocytosis of luteal cells have been observed in guinea-pigs during postpartum regression of the corpus luteum (Paavola, 1979). In the human corpus luteum, a marker antigen considered to be relatively specific for phagocytosing macrophages increased markedly as the corpus luteum aged and regressed (Takaya *et al.*, 1997). The corpus luteum regresses primarily through loss of cells by apoptosis (Juengel *et al.*, 1993; Shikone *et al.*, 1996; Rueda *et al.*, 1997), and phagocytosis by macrophages is the final event for cells dying by apoptosis (Savill *et al.*, 1993). Phagocytosis of cells may hold the important clue for the relatively benign process of luteal regression, also highlighting the critical role for macrophages. Evidence from other systems indicates that macrophages are stimulated to suppress their production of proinflammatory mediators, and to increase their production of anti-inflammatory cytokines through the act of ingesting apoptotic cells (Voll *et al.*, 1997; Fadok *et al.*, 1998; McDonald *et al.*, 1999). Fadok *et al.* (1998) reported that the recognition and ingestion of apoptotic cells by macrophages stimulates macrophage production of anti-inflammatory mediators such as transforming growth factor β 1 (TGF- β 1), prostaglandin E_2 and platelet-activating factor, while inhibiting the production of eicosanoids and certain proinflammatory cytokines, such as TNF- α and interleukins. TGF- α is proposed to have an important autocrine action in this cascade. The uptake of apoptotic cells by macrophages also inhibits the synthesis and secretion of certain chemokines, macrophage inflammatory protein 2 (Mip-2) and Mip-1 α , but not that of monocyte chemoattractant protein 1 (McDonald *et al.*, 1999). Such a mechanism might allow for the selective recruitment of monocytes, which are very prevalent in the corpus luteum in association with expression of monocyte chemoattractant protein 1 (Townson *et al.*, 1996; Penny, 2000).

The emphasis here is upon the speed and efficiency of macrophages to ingest apoptotic luteal cells and membrane-bound apoptotic cell bodies before the apoptotic cells lyse. As discussed by Savill (1997), an apoptotic cell disappears

as a histologically recognized entity in only 1–2 h as a result of the rapid ingestion and degradation by phagocytes. Even a low rate of apoptosis results in speedy loss of tissue: assuming 1% of cells in a snapshot histological section are apoptotic, and a clearance time of 1 h by phagocytes, then 25% of cells will have disappeared in 24 h (Savill, 1997). If apoptotic cells were not cleared quickly, and allowed to lyse, this would represent a necrosis scenario, in which potentially noxious cell contents released into the interstitial space potentially could lead to an inflammatory condition. If this happened in the corpus luteum, inflammatory changes would pose a peril to surrounding ovarian tissues, including the oocytes. This general concept is highlighted by Savill (1997) and Green and Beere (2000), who refer to the protective and injury-limiting potential of phagocytosis of apoptotic cells in contrast to necrosis. However, it cannot be excluded that some necrosis occurs in luteal regression, particularly in experiments in which regression is induced prematurely and acutely by injections of PGF $_{2\alpha}$ or in which luteotrophic support is withdrawn, as illustrated in reports by Rueda *et al.* (1995b) and Fraser *et al.* (1995). The experimental induction of acute, premature luteal regression might be expected to cause a sudden surfeit of dead and dying cells, before the arrival of sufficient numbers of monocytes and macrophages, leading to some necrosis. Finally, it should be noted that the hormonal milieu in the corpus luteum might be highly conducive to increased phagocytic activity by macrophages. A number of reports have highlighted the effects of steroids on macrophages (Nicol *et al.*, 1965; Vernon-Roberts, 1969; Chao *et al.*, 1995; Miller and Hunt, 1996), and the evidence indicates that oestrogen has a marked stimulatory effect on the phagocytic activity of macrophages (Nicol *et al.*, 1965; Vernon-Roberts, 1969) and that the action of oestrogen is not inhibited by progesterone (Nicol *et al.*, 1965). Higher oestrogen concentrations, which would be expected in the non-regressing corpus luteum, particularly in those species in which the corpus luteum expresses aromatase (Elbaum and Keyes, 1976; Stouffer *et al.*, 1980), may be inhibitory to the production of proinflammatory mediators in the corpus luteum, as has been reported in isolated monocytes and macrophages (Polan *et al.*, 1989; reviewed by Miller and Hunt, 1996). The oestrogen content of the corpus luteum, coupled with the presence of oestrogen receptors in macrophages (Gulshan *et al.*, 1990), represent potentially favorable conditions for anti-inflammatory activities of macrophages.

Role of immune cells in maternal recognition of pregnancy

If hypothesis 1 is correct, a signal must be present during maternal recognition of pregnancy that either reduces production of chemoattractant molecules or suppresses the response of the immune cells to them. During the oestrous cycle, lymphocytes and macrophages are recruited into the corpus luteum, which is destined to regress; this recruitment

of immune cells does not occur at the similar time of early pregnancy (Lobel and Levy, 1968; Bagavandoss *et al.*, 1990). Activation of resident immune cells might also be compromised during pregnancy, because expression of MHC molecules is lower in corpora lutea from pregnant versus non-pregnant animals (Benyo *et al.*, 1991; Kenny *et al.*, 1991). Thus, there must be a mechanism by which recruitment and activation of immune cells is suppressed during early pregnancy. As the uterus prepares to accept the fetal allograft, mechanisms to accomplish this may also influence luteal survival. Takiguchi *et al.* (2000) observed that luteal progesterone and superoxide dismutases were increased by placental supernatant. They speculated that this was a component of the rescue of the corpus luteum that occurs during maternal recognition of pregnancy. The uterus of pregnant ewes produces proteins that inhibit lymphocyte proliferation (Skopets and Hansen, 1993), but it is unknown whether these proteins travel to the ovary or whether similar proteins are produced coincidentally in the ovary. A trophoblast-derived molecule that suppresses IFN- γ -induced MHC class II expression has been described (Peyman, 1999). This molecule was also expressed in the ovary, raising the possibility that it is involved in preventing the increase in MHC expression in the corpus luteum during maternal recognition of pregnancy.

The localized immune response that is initiated at the time of luteolysis may also be prevented in early pregnancy by the high concentrations of progesterone within the corpus luteum. Progesterone is a very effective immunosuppressive agent owing to its ability to inhibit lymphocyte proliferation and function (reviewed by Siiteri and Stites, 1982; Grossman, 1984; Kelly, 1994). In addition to suppressing lymphocyte function, progesterone inhibits cytokine action on luteal cells directly. Stimulation of prostaglandin production by cytokines is completely inhibited by progesterone and, when combined with IFN- γ , progesterone can suppress cytokine-induced expression of MHC molecules on luteal cells (Pate, 1995). Thus, the decline in intraluteal progesterone concentrations initiated by uterine PGF $_{2\alpha}$ may release the immune cells from the suppressive effects of progesterone, and allow cytokines to exert their effects on luteal cells. With maintenance of progesterone during maternal recognition of pregnancy, suppression of immune cells would be sustained.

Conclusions

Immune cells are recruited into the corpus luteum as it ages and regresses, and these cells probably have important physiological roles, particularly during the regression phase. Most experiments have been conducted *in vitro* to test the hypothesis that immune cells are involved directly in inhibition of steroidogenesis and destruction of the tissue. The data from a number of reports are consistent with this hypothesis. However, more definitive experiments to test this hypothesis *in vivo* have yet to be performed. Furthermore, the results of some experiments have led to an

alternative hypothesis, that immune cells nurture the surviving luteal cells as the corpus luteum regresses as a result of apoptotic events. This may be true during luteolysis, as well as during luteinization and luteal rescue in early pregnancy. It may yet be determined that immune cells are integral components of most of the functional and structural changes that occur throughout the lifespan of the corpus luteum. Whether the role of immune cells is to support or destroy luteal cells may depend on the balance of immune cells present, their activation state, and the milieu of cytokines and hormones present at a particular time.

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