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Tumor Necrosis Factor Production and Accumulation of Inflammatory Cells in the Corpus Luteum of Pseudopregnancy and Pregnancy in Rabbits[1]

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ABSTRACT

The potential involvement of macrophages, T lymphocytes, and the cytokine tumor necrosis factor (TNF) in regression of the corpus luteum was investigated at different stages of pseudopregnancy and pregnancy by use of immunocytochemical methods and a TNF bioassay. Few macrophages (11 ± 6 per high power field of 8-μm frozen sections of corpus luteum, Day 10 of pseudopregnancy) were observed until the very end of pseudopregnancy, when the number of macrophages increased greatly (176 ± 42 per high power field, Day 19 of pseudopregnancy). Pregnancy, of 32 days duration, delayed large-scale macrophage accumulation until 3 days after parturition (154 ± 30 per high power field). Low TNF activity (~1.0 U/mg protein) was detected in incubations of luteal tissue at all stages; in response to lipopolysaccharide, TNF values in medium increased 10- to 30-fold at times of luteal regression and macrophage accumulation (1 day postpartum and Day 19 of pseudopregnancy). Class II-positive T lymphocytes were observed in luteal tissue, but unlike macrophages, the number of lymphocytes did not increase at the time of regression of the corpus luteum. These data are consistent with the hypothesis that involution of the corpus luteum is promoted through the interactions of inflammatory cells and action of TNF, although the action of TNF has not been determined in this luteal tissue. Through unknown mechanisms, pregnancy postpones the accumulation of macrophages in the corpus luteum, in association with the prolongation of luteal function until the time of parturition.

INTRODUCTION

A unique characteristic of the mammalian corpus luteum is the inherent capacity for the loss of progesterone secretion and for self-destruction, which signal the end of the luteal phase of the nonfertile menstrual or estrous cycle and permit the beginning of a new cycle. In some large domestic species, the loss of progesterone secretion at the end of the luteal phase is attributed to the action of prostaglandin $F_{2\alpha}$ produced by the uterus (McCracken et al., 1981; Thatcher et al., 1986). In other species, such as rabbits (Hilliard et al., 1974; Miller and Keyes, 1976), humans (Beling et al., 1970), and monkeys (Neill et al., 1969), the signal responsible for termination of progesterone secretion is unknown, since the presence of the uterus is not mandatory for termination of the luteal phase. Because prostaglandin $F_{2\alpha}$ has often been associated with the loss of progesterone secretion, it has been called the luteolytic hormone in certain species. But this is a simplification, since involution of the corpus luteum invariably occurs, which must involve an array of mechanisms for tissue disposal.

Little is known about the biology of the involution of the corpus luteum. Macrophages have been identified in corpora lutea of guinea pigs (Paavola, 1979), mice (Kirsch et al., 1981; Hume et al., 1984), rats (Bulmer, 1964), humans (Adams and Hertig, 1969), and cattle (Lobel and Levy, 1968). Lymphocytes have also been observed in the cow corpus luteum (Lobel and Levy, 1968). Recently, we reported that macrophages and T lymphocytes are present in the regressing corpus luteum of the rabbit, and tumor necrosis factor-alpha (TNF-α) a monokine of macrophage origin, is also present (Bagavandoss et al., 1988). The immune system may play an important role in ovarian function. Autoimmune diseases are often associated with premature ovarian failure (Anderson et al., 1968; Moraes Ruehsen

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et al., 1972) and this has been related to the increased expression of major histocompatibility antigens on leukocytes (Walfish et al., 1983). Neonatal thymectomy results in dysgenesis of ovarian follicular development (Sakakura and Nishizuka, 1972; Lintern-Moore, 1977), and treatment of adult rats with antithymocyte serum results in anovulation and persistence of existing corpora lutea (Bukovsky et al., 1977), leading to the proposal that the immune system may participate in the termination of the lifespan of the corpus luteum (Bukovsky and Presl, 1981).

We have proposed that regression of the corpus luteum might be mediated through the activities and interrelations of macrophages, lymphocytes, and their cytokine products (Bagavandoss et al., 1988). Here we have examined this concept more strictly by determining the chronology of T lymphocyte and macrophage infiltration and associated TNF activity in regressing and nonregressing corpora lutea of the same ages, i.e., in the corpora lutea of pseudopregnancy and pregnancy, respectively.

MATERIALS AND METHODS

Animals

Sexually mature New Zealand White rabbits weighing 3.5 – 4 kg were maintained at 22°C with 12L:12D, and fed 3.8 oz (106.4 g) rabbit chow daily (Lab Chows, Purina Mills, Inc., St. Louis, MO).

Estrous female rabbits were mated either with a vasectomized male to induce pseudopregnancy or with a fertile male; the day of mating was designated Day 0. On selected days of pseudopregnancy and pregnancy, and on Days 1 and 3 postpartum, the rabbits (5 rabbits each day; total of 60 animals) were anesthetized with sodium pentobarbital (165 mg/2.5 ml) and the corpora lutea were removed aseptically for incubation (see below). Pseudopregnancy lasts about 19 days; i.e. serum progesterone values have returned to estrous levels by Day 19 postovulation (Browning et al., 1980; Keyes et al., 1983). Parturition occurs on Day 32 or 33 postovulation (Keyes et al., 1983).

Immunofluorescence and Immunocytochemistry

Ovaries were removed and either frozen in O.C.T. Compound (Lab-Tek Products, Naperville, IL) for cryostat sections or fixed in 1% paraformaldehyde for 4 h at 4°C, processed as described previously (Bagavandoss et al., 1988; Beckstead, 1985), and embedded in paraffin.

For immunofluorescence, cryostat sections (8 μm) were fixed in acetone for 15 min at 25°C, rinsed in phosphate-buffered saline (PBS) containing bovine serum albumin (BSA, 0.1%) for 5 min, and incubated with normal goat serum (10%) for 30 min at 25°C. After blotting excess serum, the sections were incubated with specific monoclonal antibodies against rabbit T lymphocytes (Wilkinson et al., 1984), macrophages (Tsukada et al., 1986), or class II antigen (Lobel and Knight, 1984) for 30 min at 25°C. The RAM 11 anti-macrophage antibody was a generous gift of Dr. A. Gown, University of Washington, Seattle, WA. Subsequently, the sections were rinsed in PBS-BSA, and incubated with either rhodamine or fluorescein-conjugated goat anti-mouse IgG (Cappel, Malvern, PA) as above, then rinsed in PBS-BSA, and mounted for viewing. Nonspecific binding was determined in the absence of primary antibody or in the presence of monoclonal antibodies raised against other antigens or in the presence of nonimmune gamma globulin. For quantification, all samples were randomly coded and the cells were enumerated in five random fields in each of 1 to 4 corpora lutea per rabbit with a 40× objective and expressed as number of cells per high power field. The mean number of cells per high power field obtained from the corpora lutea of one rabbit represents n = 1.

For double labeling of T lymphocytes, the cells were labeled with two different monoclonal antibodies by the following procedures. After incubation with first primary antibody (against T cell antigen), sections were incubated with rhodamine-conjugated goat anti-mouse antibody, rinsed in PBS-BSA, and then incubated with excess of unlabeled goat anti-mouse IgG for 30 min at 25°C to block all the unbound sites on the first primary antibody. The sections were then rinsed in PBS-BSA and exposed to the second primary antibody (against class II antigen), rinsed, incubated with fluorescein-conjugated goat anti-mouse IgG, rinsed again and mounted as described above. Specificity of the double labeling was determined in the absence of second primary antibody.

For immunocytochemistry, sections were incubated with goat serum and primary antibody or nonspecific antibodies as described above. Subsequently, the sections were rinsed in PBS-BSA and stained with avidin-biotin-peroxidase complex according to the manufacturer's instructions (Vector, Burlingame, CA; DAKO Corp., Santa Barbara, CA).

Preparation of Corpus Luteum-Conditioned Medium

Corpus luteum-conditioned medium was prepared as described previously (Bagavandoss et al., 1988). Briefly, the interstitial tissue was dissected off the corpora lutea under a dissecting microscope and the corpora lutea were then sliced in 1 ml of serum-free medium 199 (GIBCO, Grand Island, NY) containing gentamicin (50 μg/ml) and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES, 10 mM) and incubated for 30 min in sterile tubes at 37°C in an atmosphere of 95% air:5% CO_2 in the absence or in the presence of lipopolysaccharide (LPS, 10 μg/ml; Sigma Chemical Co., St. Louis, MO). The tubes were tightly capped, transferred to a water bath, and incubated at 37°C with gentle shaking for an additional 4 h. After incubation, the tubes were centrifuged (800 × *g*) and the supernatants (conditioned medium) were aspirated and filtered through 0.22-μm filters (Millipore, Bedford, MA) and were kept frozen at −70°C.

The tissue pellets were homogenized and protein was measured with a commercial kit (Pierce Chemical, Rockford, IL) as described previously (Redinbaugh and Turley, 1986).

TNF Assay

A highly TNF-sensitive mouse fibrosarcoma cell line (WEHI 164 clone 13) was obtained from Dr. A. Waage, University of Trondheim, Norway. Using these cells, we measured the activity of TNF-α in conditioned media according to procedures of Espevik and Nissen-Meyer (1986). Briefly, the WEHI 164 cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum, 1 mM L-glutamine, and 20 μg/ml gentamicin (Sigma Chemical Co) at 37°C in an atmosphere of 95% air:5% CO_2.

For bioassay, the cells were aliquoted into microtiter plates (50,000 cells/100 μl/well) in the RPMI 1640 medium containing gentamicin, glutamine, actinomycin D (0.1 μg/ml), and 1% fetal bovine serum. Serial dilutions of human recombinant TNF standards (1×10^7 U/mg; kindly provided by Dr. Leo Lin, Cetus Corp, Emeryville, CA) and corpora lutea-conditioned medium (100 μl/well) were added to the wells and incubated as above for 20 h. Subsequently, the cytotoxicity of TNF on WEHI cells was indirectly visualized by the addition of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co.), as described by Mossman (1983). The tetrazolium ring in MTT is cleaved in active mitochondria, thus, blue formazan granules are found only in living cells. MTT (20 μl; 5 mg/ml PBS) was added to each well and the cells were incubated for an additional 4 h. Subsequently, 100 μl of the medium was aspirated and 100 μl of warm (37°C) isopropanol containing 0.04 N hydrochloric acid was added to the wells to dissolve the blue formazan crystals. The plates were kept at 25°C overnight and read on a multiwell spectrophotometer at 550 nm. The assay was sensitive to 0.01 units of recombinant human TNF-α.

Neutralization of TNF Activity with Antibody

To evaluate the specificity of the bioassay for TNF, 300 μl of corpus luteum-conditioned medium was mixed with either 100 μl of goat antibody against human recombinant TNF-α or 100 μl of nonimmune goat globulin, incubated for 1 h at 37°C, and subsequently assayed. This antibody neutralizes rabbit TNF (Mathison et al., 1988) and was provided by Dr. J. C. Mathison of Scripps Clinic, La Jolla, CA.

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA), and significant differences between groups were subsequently established by Scheffe's test: a p value of <0.05 was taken as the significance level. Significance between TNF-activity in the presence or absence of LPS was determined by paired t-test. All values represent the mean ± SEM of determinations from five rabbits.

RESULTS

Qualitative Distribution of Lymphocytes and Macrophages

By immunofluorescence and immunocytochemistry, T lymphocytes and macrophages were observed in the rabbit ovary. The T lymphocytes were present at all stages of pseudopregnancy, pregnancy, and the postpartum periods and were localized primarily within the corpus luteum. They were distributed near blood vessels and in intercellular spaces of the corpora lutea. Although T lymphocytes were observed mostly in aggregates, they were also distributed individually (Fig. 1). To determine if these T lymphocytes were activated (Greaves et al., 1979: Brown et al., 1984), we double-labeled the cells with antibodies against both T cell and

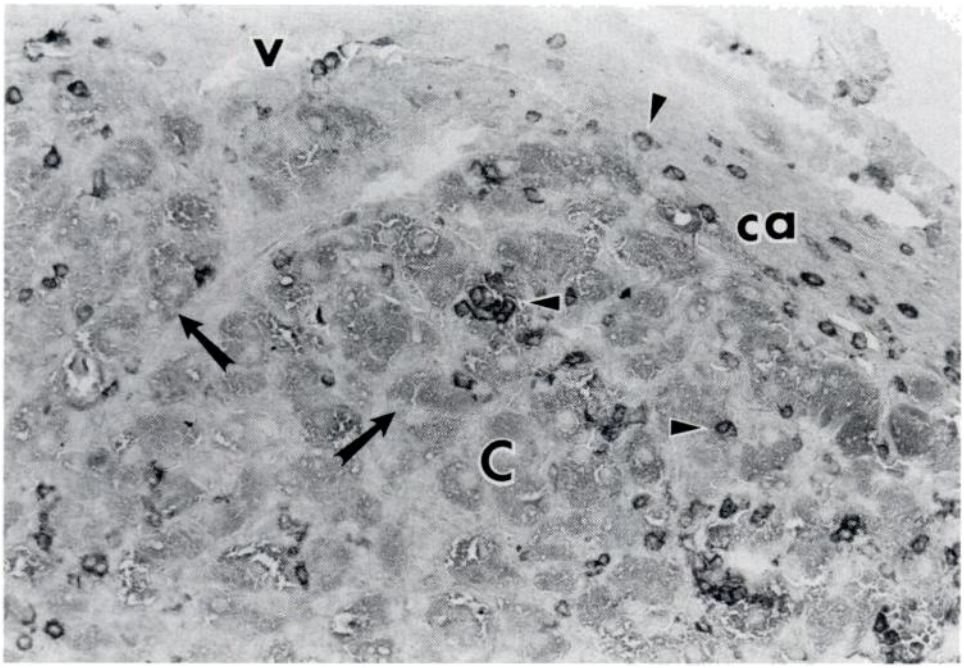

FIG. 1. Immunohistochemical localization of T lymphocytes in a section of a corpus luteum *(C)* from a Day 5 pseudopregnant rabbit. The T lymphocytes *(arrowheads)* are distributed individually and in clusters among the luteal cells *(arrows)*, along the vessels *(V)*, and along the capsule *(ca)* of the corpus luteum (×188).

class II antigens. Class II antigen on the T lymphocytes was detected as early as Day 5 postovulation (Fig. 2).

The distribution of macrophages was similar to that of T lymphocytes in that macrophages were localized predominantly within the corpus luteum. However, the temporal pattern was not the same. Macrophages were most evident in the regressing corpus luteum, whereas the T lymphocytes were present in the corpus luteum at all stages examined. Thus, corpora lutea of Day 5 pseudopregnancy and pregnancy and Day 19 pregnancy had very few macrophages (Fig. 3A), whereas the regressing corpora lutea of Day 19 pseudopregnancy and Day 3 postpartum had strikingly large numbers of macrophages (Fig. 4B). In addition, T lymphocytes and macrophages were also present in atretic follicles of the ovary (not shown).

Quantitative Distribution of T Lymphocytes and Macrophages

Using immunoflourescence, we counted T lymphocytes and macrophages. The number of T lymphocytes did not vary significantly among different days of pseudopregnancy, pregnancy, and postpartum (Fig. 4). Although macrophages were observed in the corpus luteum at all stages, relatively few were observed until the time of regression. In pseudopregnancy, the numbers of macrophages remained relatively low through Days 10 – 15 (11 ± 6 per high power field) and increased significantly by Day 19 (176 ± 42; Fig. 4A). In pregnant rabbits, a significant increase in the number of macrophages was not observed until Day 3 postpartum (154 ± 30; Fig. 4B).

TNF Production by the Corpus Luteum

In the absence of lipopolysaccharide, low TNF activity (~1.0 U/mg protein) was detectable in medium of incubated corpora lutea from all stages of pseudopregnancy and pregnancy (Fig. 5). In the presence of lipopolysaccharide, TNF accumulation in medium was greatest on Day 19 of pseudopregnancy at a time when the number of macrophages were high (Fig. 5). In corpora lutea of Day 5 pregnancy, Day 5 pseudopregnancy, and Day 19 pregnancy, TNF production was enhanced 2- to 4-fold in response to lipopolysaccharide. By contrast, in the corpora lutea of Day 1 postpartum and in the regressing corpora lutea of Day 19 pseudo-

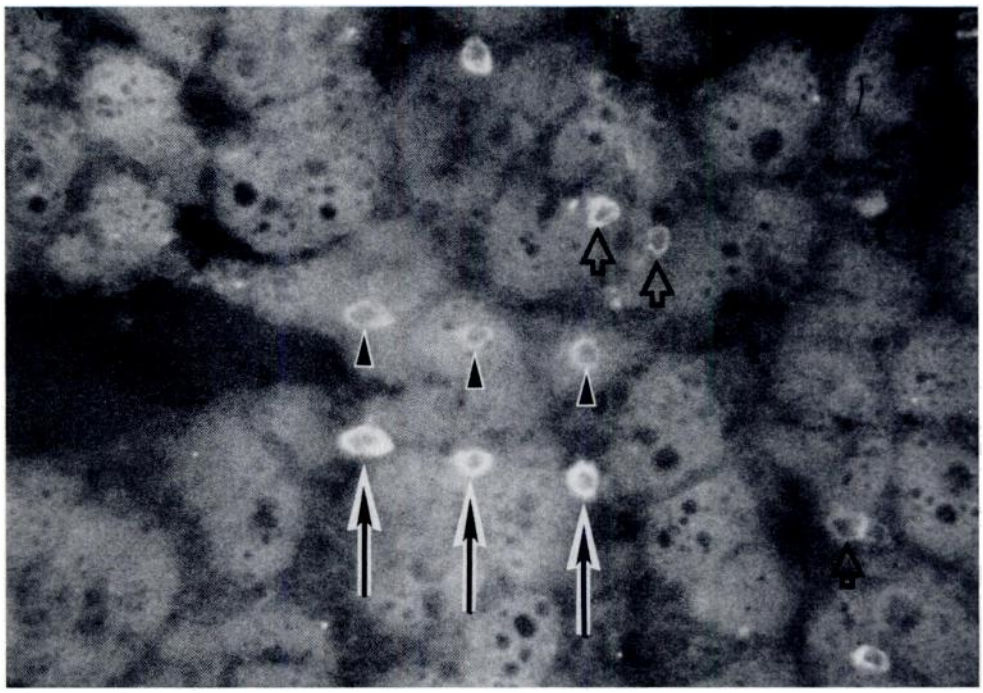

FIG. 2. Immunofluorescence of lymphocytes double-labeled with antibodies against T cell (rhodamine label) and class II (fluorescein label) antigens in a section of a corpus luteum from a Day 5 pregnant rabbit. The section was first photographed with a rhodamine filter *(arrows)*; subsequently, the section was slightly moved and reexposed with a fluorescein filter *(arrowheads)* on the same frame to demonstrate the antigens on the same cells. Note that some T lymphocytes *(short arrows)* are not class II-positive (×400).

pregnancy, TNF production was stimulated respectively 10- and 30-fold by lipopolysaccharide (Fig. 5). Unconditioned medium (no cells) ± LPS had no activity in the bioassay.

The specificity of the bioassay for TNF was confirmed by incubating corpus luteum-conditioned media with either an antibody raised against human TNF or with nonimmune goat globulin. TNF activity was reduced by 95% in the presence of TNF antibody, both in LPS-stimulated samples and in samples without LPS (Table 1).

DISCUSSION

The chronology of macrophage and T lymphocyte infiltration into the corpus luteum reveals that both types of cells are present throughout the luteal phase, although the numbers of macrophages increase greatly at the end of pseudopregnancy and after parturition. The presence of T lymphocytes, which are class II-positive, suggests that these cells are activated (Winchester and Kunkel, 1979) and therefore capable of activating macrophages (Pels et al., 1984). Thus, the potential exists for interactions between macrophages and T lymphocytes, mediated by monokines and lymphokines (Pels et al., 1984). Also the potential exists for these cells, via production of cytokines, to influence various functions of the differentiating corpus luteum, such as angiogenesis (Polverini and Leibovich, 1984; Kaminski and Auerbach, 1988), or progesterone secretion as reported in granulosa cells, follicles, or corpora lutea (Kirsch et al., 1981; Halme et al., 1985; Emoto and Baird, 1988; Fukuoka et al., 1988, 1989; Roby and

TABLE 1. Neutralization of TNF activity[a] in corpus luteum-conditioned medium by goat anti-human recombinant TNF-α antibody.*

	Plus LPS[b]	No LPS
Goat anti-human TNF antibody	4[a]	<0.01
Goat non-immune globulin	87	0.07

[a]TNF units per mg of luteal tissue protein (see text for details of TNF bioassay).

[b]LPS: lipopolysaccharide. Luteal tissue was incubated with or without LPS (see text for details of incubation).

*Data are from a single experiment.

Terranova, 1988; Adashi et al., 1989), ultimately delimiting the lifespan of the corpus luteum through cytocidal activity and phagocytosis (Paavola, 1979).

The major increase in the number of macrophages occurs only in corpora lutea entering terminal stages of regression, either at the end of pseudopregnancy or several days after parturition. This increase may be due to either proliferation of existing macrophages or infiltration of monocytes or both. At these stages, progesterone secretion has either declined markedly as in pseu-

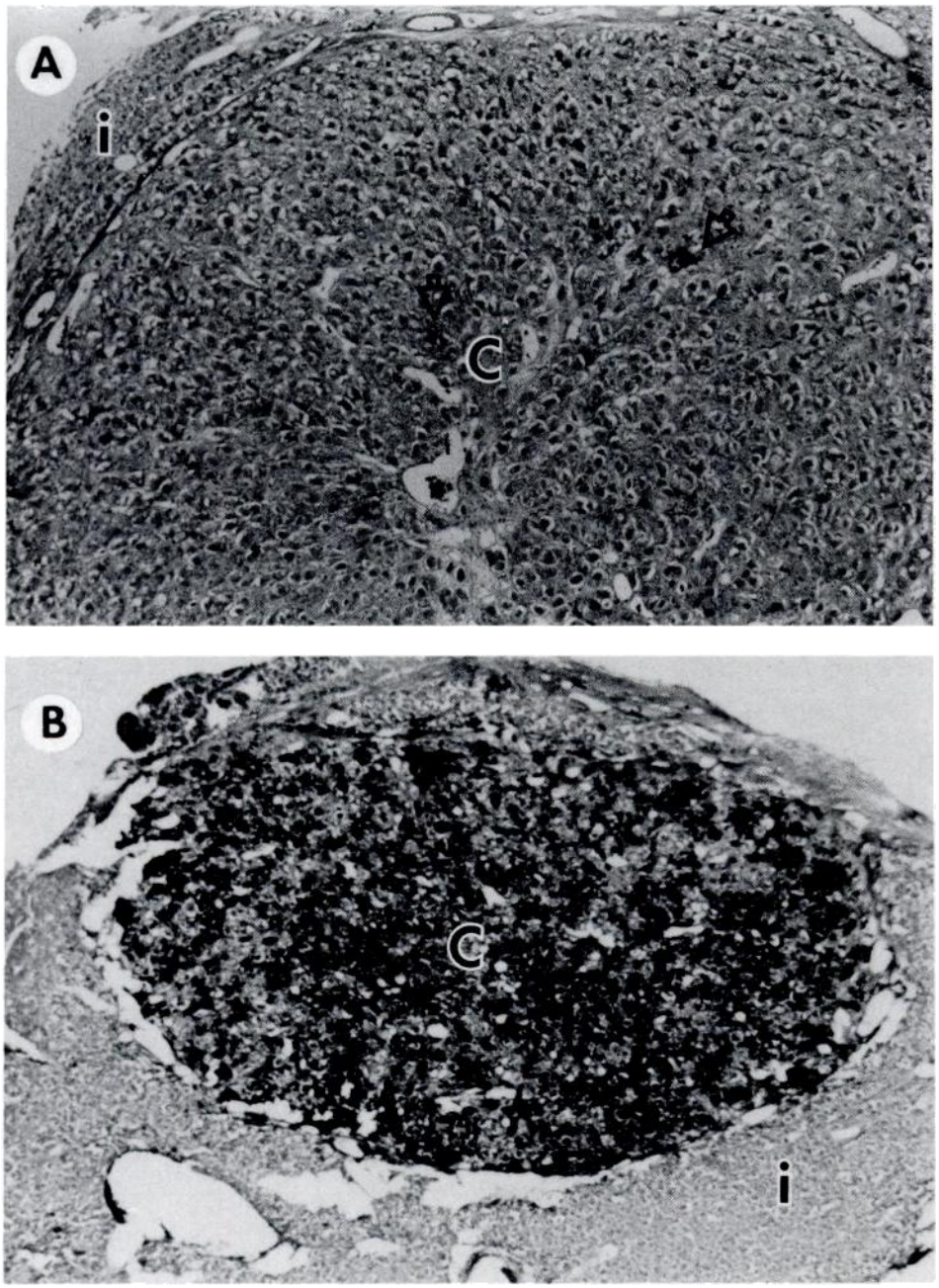

FIG. 3. Immunohistochemical localization of macrophages in a section of corpus luteum, *C*, from Day 19 pregnant and Day 19 pseudopregnant rabbits. *(A)* In the pregnant rabbit, only a few scattered macrophages *(arrows)* were observed within the corpus luteum (×38). With the exception of Days 1 and 3 postpartum and Day 19 of pseudopregnancy, a similar distribution of macrophages was observed at other stages of pregnancy and pseudopregnancy (not shown). *(B)* In the Day 19 pseudopregnant rabbit, the corpus luteum, *C*, is heavily infiltrated with macrophages *(black deposits)*. Note that the interstitium, *i*, is devoid of macrophages. (×51). Although not shown here, a similar distribution of macrophages was observed in corpora lutea 3 days postpartum.

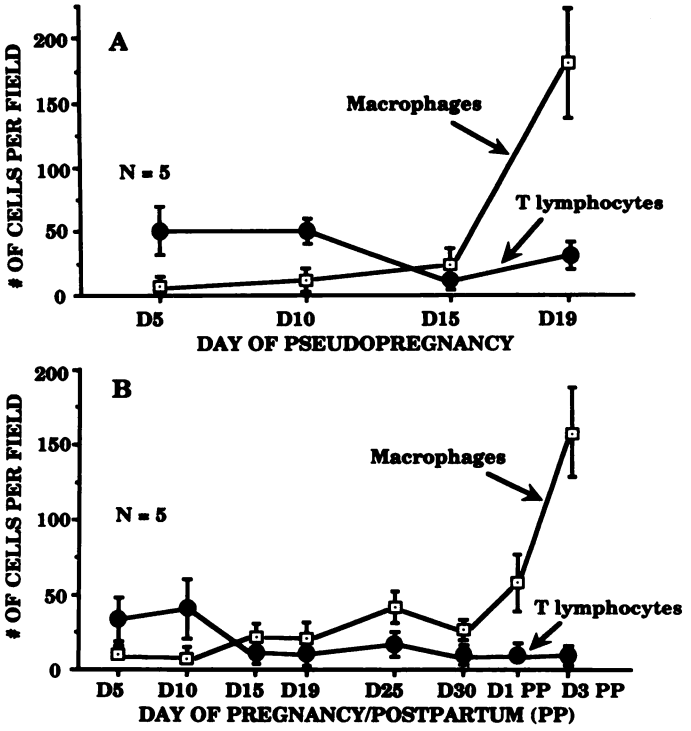

FIG. 4. Quantification of macrophages and T lymphocytes in the corpora lutea of pseudopregnancy *(A)* and pregnancy *(B)*. T lymphocytes were present as early as Day 5 of pseudopregnancy and pregnancy, with no detectable significant changes in numbers of these cells at other stages of pseudopregnancy or pregnancy. In contrast, during involution of the corpus luteum, the numbers of macrophages increased dramatically as observed on Day 19 of pseudopregnancy ($p<0.01$) and Day 3 postpartum ($p<0.01$). Mean ± SEM; n = 5 rabbits at each stage of pseudopregnancy and pregnancy (total of 60 animals).

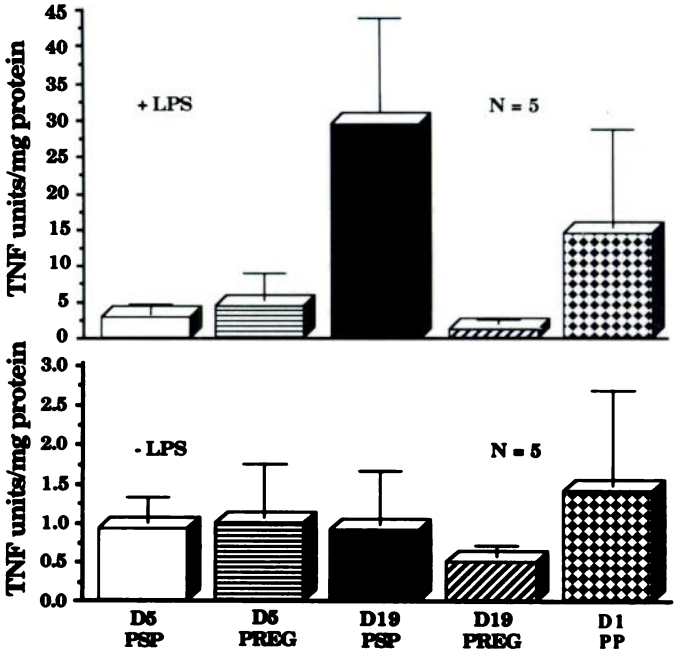

FIG. 5. TNF production by luteal tissue incubated with or without lipopolysaccharide (LPS). Corpora lutea were removed from pseudopregnant (PSP), pregnant (PREG), and postpartum (PP) rabbits. In the absence of LPS, low but detectable TNF activity was observed *(bottom panel)*. Upon stimulation with LPS, the nonregressing corpora lutea on Day 5 of pseudopregnancy and on Days 5 and 19 of pregnancy produced only 2- to 4-fold TNF *(top panel)*. However, a 10- to 30-fold increase in activity was observed respectively in the regressing corpora lutea on Day 1 postpartum and Day 19 pseudopregnancy ($p<0.05$). Mean ± SEM; n = 5 rabbits each day; tissues in *top* (+LPS) and *bottom* (– LPS) *panels* are from same animals.

dopregnancy or essentially ceased as in the postpartum period (Keyes et al., 1983). Thus, it is tempting to speculate that as a result of diminished rates of steroidogenesis and through the production of chemotactic signals, monocytes infiltrate into the luteal tissue where they become activated macrophages. Progesterone has biphasic effects on the macrophage (Polan et al., 1988b): at low concentration, it stimulates interleukin-1 production, whereas at high concentrations, which would prevail in the luteal tissue, progesterone can inhibit the production of interleukin-1 at the level of transcription (Polan et al., 1988a). Progesterone also inhibits other metabolic activities of monocytes, macrophages, and T lymphocytes (Siiteri and Stites, 1982; Stites and Siiteri, 1983; Schreiber et al., 1988). As luteal progesterone secretion decreases, the circulating monocytes may become more responsive to chemotactic signals, and the corpus luteum-associated macrophages and lymphocytes may secrete, respectively, increasing concentrations of monokines such as interleukin-1 and TNF, and lymphokine, such as gamma interferon.

Although no increase in basal TNF production was observed in incubated corpora lutea of late pseudopregnancy, the possibility exists that the TNF might be in the membrane-bound form (Kriegler et al., 1988), which escaped detection by the assay. The 10- to 30-fold increase in TNF production in response to lipopolysaccharide probably reflects the increased numbers of macrophages in luteal tissue but may also be attributable to the highly activated state of the macrophage in the microenvironment of the regressing corpus luteum. Monokines and lymphokines are chemotactic signals for monocytes (Ming et al., 1987), and TNF also induces the synthesis on granulocyte-macrophage colony-stimulating factor (Munker et al., 1986), which is a potent stimulator of monocyte proliferation and differentiation (Metcalf, 1985). Therefore, the potential exists for multiple mechanisms that promote the accumulation of macrophages in the corpus luteum.

In rabbits, the corpus lutem is the source of progesterone throughout pregnancy (Holt and Ewing, 1974), and is maintained through the combined actions of estradiol and a factor of placental origin (Holt and Ewing, 1974; Gadsby et al., 1983). Associated with extended luteal function in the second half of pregnancy is the absence of overt macrophage infiltration into the corpus luteum. The comparison of the macrophage populations in corpora lutea on Day 19 of pseudopregnancy and pregnancy indicates that their accumulation in the corpus luteum is not determined strictly by the age of the corpus luteum, but rather by other factors. The products of conception might act directly to inhibit the activation of macrophages and/or the entry of the monocytes into the corpus luteum. For example, alpha-fetoprotein, a protein synthesized by the fetus, is a potent inhibitor of the immune response (Murgita and Tomasi, 1975; Lu et al., 1984). Alternatively, the luteotropic milieu of pregnancy might prevent macrophage accumulation indirectly, by maintaining the corpora lutea that continue to synthesize progesterone.

The mechanism and pathways through which macrophages, T lymphocytes, and their cytokines might destroy the corpus luteum are largely unknown. TNF has a number of effects in different tissues (Beutler and Cerami, 1988). TNF and interkeukin-1 have inhibitory effects on steroidogenesis in cultured granulosa cells without accompanying cytocidal effects (Emoto and Baird, 1988; Fukuoka et al., 1988, 1989; Gottschall et al., 1988; Adashi et al., 1989), suggesting the potential for these cytokines to inhibit luteal steroidogenesis as well. At some doses, TNF has also been reported to stimulate follicular steroidogenesis (Roby and Terranova, 1988); in the presence of macrophages, progesterone production by mouse luteal cells (Kirsch et al., 1981) or human granulosa-luteal cells (Halme et al., 1985) is increased. The monokine, TNF, affects not only tumors (Carswell et al., 1975), but also normal cells such as fibroblasts (Hass et al., 1985) and endothelial cells (Nawroth et al., 1986). TNF has been shown to have both angiogenic (Leibovich et al., 1987) and angiostatic (Sato et al., 1987) effects, suggesting the possibility of differential effects of this monokine early in luteal development when angiogenesis is extensive (Gospodarowicz and Thakral, 1978), and at the time of regression when loss of endothelial cells is predominant (Azmi and O'Shea, 1984). Although major histocompatibility (MHC) antigens can be induced by lymphokines (Fairchild and Pate, 1989a) and TNF (Leeuwenberg et al., 1987), this induction can be inhibited by cyclic adenosine 3′, 5′-monophosphate (cAMP) (Frohman et al., 1988). As the corpus luteum ages, cAMP production by luteal cells, in response to luteinizing hormone, significantly decreases (Miller et al., 1986). Thus, although lymphocytes and macrophages are present in the developing and regressing corpus luteum, cytokine-induced expression of the MHC antigens might be greatest late in the luteal phase and during regression (Fairchild and Pate, 1989b). Thus luteal or other cells in the corpus luteum may become susceptible to recognition as "non-self" and then be destroyed through the combined activities of macrophages and T lymphocytes. The ultimate structural involution may be due to phagocytosis by macrophages, which has been observed by electron microscopy (Paavola, 1979).

In summary, these results are consistent with a proposed role for T lymphocytes and macrophages in the involution of the corpus luteum through mechanisms that remain to be elucidated, but that might resemble an immune response. The absence of macrophage infiltration in corpora lutea of pregnancy suggests a direct inhibition of monocyte entry or of their differentiation by factors associated with the conceptus, or an indirect effect by the conceptus to sustain luteal function, thereby preventing macrophage accumulation and activation.

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REFERENCES

Adams EC, Hertig AT, 1969. Studies on the human corpus luteum: I. observations on the ultrastructure of development and regression of the luteal cells during the menstrual cycle. J Cell Biol 41:696 – 715

Adashi EY, Resnick CE, Croft CS, Payne DW, 1989. Tumor necrosis factor alpha inhibits gonadotropin hormonal action in nontransformed ovarian granulosa cells. A modulatory noncytotoxic property. J Biol Chem 264: 11591 – 97

Anderson JR, Goudie RB, Gray K, Stuart-Smith DA, 1968. Immunological features of idiopathic Addison's disease: an antibody to cells producing steroid hormones. Clin Exp Immunol 3:107 – 17

Azmi TI, O'Shea JD, 1984. Mechanism of deletion of endothelial cells during regression of the corpus luteum. Lab Invest 51:206 – 17

Bagavandoss P, Kunkel SL, Wiggins RC, Keyes PL, 1988. Tumor necrosis factor-α (TNF-α) production and localization of macrophages and T lymphocytes in the rabbit corpus luteum. Endocrinology 122:1185 – 87

Beckstead JH, 1985. Optimal antigen localization in human tissues using aldehyde-fixed plastic-embedded sections. J Histochem Cytochem 33: 954 – 58

Beling CG, Marcus SL, Markham SM, 1970. Functional activity of the corpus luteum following hysterectomy. J Clin Endocrinol Metab 30:30 – 39

Beutler B, Cerami A, 1988. Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. En-

docr Rev 9:57 – 66
Brown MF, Cook RG, Van M, Rich RR, 1984. Cloned human T cells synthesize Ia molecules and function as antigen presenting cells. Hum Immunol 11: 219 – 28
Browning JY, Keyes PL, Wolf RC, 1980. Comparison of serum progesterone, 20α-dihydroprogesterone, and estradiol-17β in pregnant and pseudopregnant rabbits: evidence for postimplantation recognition of pregnancy. Biol Reprod 23:1014 – 19
Bukovsky A, Presl J, 1981. Control of ovarian function by the immune system: commentary on the criticisms of Schenzle. Med Hypotheses 7:1 – 14
Bukovsky A, Presl J, Krabec Z, Bednarik T, 1977. Ovarian function in adult rats with antithymocyte serum. Experientia 33:280 – 81
Bulmer D, 1964. The histochemistry of ovarian macrophages in the rat. J Anat 98:313 – 19
Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B, 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666 – 70
Emoto N, Baird A, 1988. The effect of tumor necrosis factor/cachectin on follicle stimulating hormone-induced aromatase activity in cultured rat granulosa cells. Biochem Biophys Res Commun 153:792 – 98
Espevik T, Nissen-Meyer J, 1986. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Methods 95:99 – 105
Fairchild DL, Pate JL, 1989a. Interferon-γ induction of major histocompatibility complex antigens on cultured bovine luteal cells. Biol Reprod 40: 453 – 57
Fairchild DL, Pate JL, 1989b. MHC antigen expression on bovine luteal cells varies during the estrous cycle. Biol Reprod 40 (Suppl. 1): 126 (Abstr.)
Frohman EM, Vayuvegula B, Gupta S, van den Noort S, 1988. Norepinephrine inhibits gamma interferon-induced major histocompatibility class II (Ia) antigen expression on cultured astrocytes via β-$_2$ adrenergic signal transduction mechanisms. Proc Natl Acad Sci USA 85:1292 – 96
Fukuoka M, Mori T, Taii S, Yasuda K, 1988. Interleukin-1 inhibits luteinization of porcine granulosa cells in culture. Endocrinology 122:367 – 69
Fukuoka M, Yasuda K, Taii S, Takakura K, Mori T, 1989. Interleukin-1 stimulates growth and inhibits progesterone secretion in cultures of porcine granulosa cells. Endocrinology 124:884 – 90
Gadsby JE, Keyes PL, Bill II CH, 1983. Control of corpus luteum function in the pregnant rabbit: role of estrogen and lack of a direct luteotropic role of the placenta. Endocrinology 113:2255 – 62
Gospodarowicz D, Thakral KK, 1978. Production of a corpus luteum angiogenic factor responsible for proliferation of capillaries and neovascularization of the corpus luteum. Proc Natl Acad Sci USA 75:847 – 51
Gottschall PE, Katsuura G, Dahl RR, Hoffmann ST, Arimura A, 1988. Discordance in the effects of interleukin-1 on rat granulosa cell differentiation induced by follicle-stimulating hormone or activators of adenylate cyclase. Biol Reprod 39:1074 – 85
Greaves MF, Verbi W, Festenstein H, Papasteriadis C, Jaraquemada D, Hayward A, 1979. "Ia-like" antigens on human T cells. Eur J Immunol 9: 356 – 62
Halme J, Hammond MG, Syrop CH, Talbert LM, 1985. Peritoneal macrophages modulate human granulosa-luteal cell progesterone production. J Clin Endocrinol Metab 61:912 – 16
Hass PE, Hotchkiss A, Mohler M, Aggarwal BB, 1985. Characterization of specific high affinity receptors for human tumor necrosis factor on mouse fibroblasts. J Biol Chem 260:12214 – 18
Hilliard J, Scaramuzzi RJ, Penardi R, Sawyer CH, 1974. Serum progesterone levels in hysterectomized pseudopregnant rabbits. Proc Soc Exp Biol Med 145:151 – 53
Holt JA, Ewing LL, 1974. Acute dependence of ovarian progesterone output on the presence of placentas in 21-day pregnant rabbits. Endocrinology 94: 1438 – 44
Hume DA, Halpin D, Charlton H, Gordon S, 1984. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80:macrophages of endocrine organs. Proc Natl Acad Sci USA 81:4174 – 77
Kaminski M, Auerbach R, 1988. Angiogenesis induction by CD-4 positive lymphocytes. Proc Soc Exp Med 188:440 – 43
Keyes PL, Gadsby JE, Yuh K-CM, Bill II CH, 1983. The corpus luteum. Int Rev Physiol 27:57 – 97
Kirsch TM, Friedman AC, Vogel RL, Flickinger GL, 1981. Macrophages in corpora lutea of mice: characterization and effects on steroid secretion. Biol Reprod 25:629 – 38
Kriegler M, Perez C, DeFay K, Albert I, Lu SD, 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 53:45 – 53
Leeuwenberg JFM, van Damme J, Jeunhomme GMAA, Buurman WA, 1987. Interferon β1, an intermediate in the tumor necrosis factor-α-induced increased MHC class I expression and an autocrine regulator of the constitutive MHC class I expression. J Exp Med 166:1180 – 85
Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N, 1987. Macrophage-induced angiogenesis is mediated by tumor necrosis factor-α. Nature 329:630 – 32
Lintern-Moore S, 1977. Effect of athymia on the initiation of follicular growth in the rat ovary. Biol Reprod 17:155 – 61
Lobel SA, Knight KL, 1984. The role of rabbit Ia molecules in immune functions as determined with the use of an anti-Ia monoclonal antibody. Immunology 51:35 – 43
Lobel BL, Levy E, 1968. Enzymatic correlates of development, secretory function and regression of follicles and corpora lutea in the bovine ovary II. Formation, development and involution of corpora lutea. Acta Endocrinol 59 (Suppl. 132):35 – 51
Lu CY, Changelian PS, Unanue ER, 1984. α-Fetoprotein inhibits macrophage expression of Ia antigens. J Immunol 132:1722 – 27
Mathison JC, Wolfson E, Ulevitch RJ, 1988. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Invest 81:1925 – 37
McCracken JA, Schramm W, Barcikowski B, Wilson L Jr, 1981. The identification of prostaglandin F2-α as a uterine luteolytic hormone and the hormonal control of its synthesis. Acta Vet Scand (Suppl.) 77:71 – 88
Metcalf D, 1985. The granulocyte-macrophage colony-stimulating factors. Science 229:16 – 22
Miller JB, Keyes PL, 1976. A mechanism for the regression of the rabbit corpus luteum: uterine-induced loss of luteal responsiveness to 17β-estradiol. Biol Reprod 15:511 – 18
Miller JB, LaBarbera AR, Hunzicker-Dunn M, 1986. Estradiol suppression of luteinizing hormone (LH)/human chorionic gonadotropin receptors and LH-sensitive adenylyl cyclase without decreased adenosine 3′, 5′-monophosphate content in rabbit corpora lutea. Endocrinology 118:2016 – 23
Ming WJ, Bersan L, Mantovani A, 1987. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J Immunol 138: 1469 – 74
Moraes Ruehsen M de, Blizzard RM, Garcia-Bunuel R, Jones GS, 1972. Autoimmunity and ovarian failure. Am J Obstet Gynecol 112:693 – 703
Mossman T, 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55 – 63
Munker R, Gasson J, Ogawa M, Koeffler HP, 1986. Recombinant human TNF induces production of granulocyte-monocyte colony-stimulating factor. Nature 323:79 – 82
Murgita RA, Tomasi TB, 1975. Suppression of the immune system by α-fetoprotein. I. The effect of mouse α-fetoprotein on the primary and secondary antibody response. J Exp Med 141:269 – 86
Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D, 1986. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J Exp Med 163:1363 – 75
Neill JD, Johansson EDB, Knobil E, 1969. Failure of hysterectomy to influence the normal pattern of cyclic progesterone secretion in the rhesus monkey. Endocrinology 84:464 – 65
Paavola LG, 1979. Cellular mechanisms involved in luteolysis. Adv Exp Med Biol 112:527 – 33
Pels E, De Weger RA, Den Otter W, 1984. Lymphocyte induced macrophage toxicity: characterization of the macrophage cytotoxicity-inducing lymphocyte. Immunobiology 166:84 – 95
Polan ML, Carding S, Loukides J, 1988a. Progesterone modulates interleukin-1 (IL-1) m-RNA production by human pelvic macrophages. Fertil Steril 50: S4 (Abstr.)
Polan ML, Daniele A, Kuo A, 1988b. Gonadal steroids modulate human monocyte interkeukin-1 (IL-1) activity. Fertil Steril 49:964 – 68
Polverini PJ, Leibovich SJ, 1984. Induction of neovascularization in vivo and endothelial proliferation in vitro by tumor-associated macrophages. Lab Invest 51:635 – 47
Redinbaugh MG, Turley RB, 1986. Adaptation of the bicinchoninic acid protein assay for use with microtiter plates and sucrose gradient fractions. Anal Biochem 153:267 – 71
Roby KF, Terranova P, 1988. Tumor necrosis factor alpha alters follicular ster-

oidogenesis *in vitro*. Endocrinology 123:2952 – 54

Sakakura T, Nishizuka Y, 1972. Thymic control mechanism in ovarian development: reconstitution of ovarian dysgenesis in thymectomized mice by replacement with thymic and other lymphoid tissues. Endocrinology 90: 431 – 37

Sato N, Fukuda K, Nariuchi H, Sagara N, 1987. Tumor necrosis factor inhibiting angiogenesis in vitro. J Natl Cancer Inst 79:1383 – 91

Schreiber AD, Nettl FM, Sanders MC, King M, Szabolcs P, Friedman D, Gomez F, 1988. Effect of endogenous and synthetic sex steroids on the clearance of antibody-coated cells. J Immunol 141:2959 – 66

Siiteri PK, Stites DP, 1982. Immunologic and endocrine interrelationships in pregnancy. Biol Reprod 26:1 – 14

Stites DP, Siiteri PK, 1983. Steroids as immunosuppressants in pregnancy. Immunol Rev 75:117 – 38

Thatcher WW, Bazer FW, Sharp DC, Roberts RM, 1986. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pigs and horses. J Anim Sci 62 (Suppl. 2): 25 – 46

Tsukada T, Rosenfeld M, Ross R, Gown AM, 1986. Immunocytochemical analysis of cellular components in atherosclerotic lesions: use of monoclonal antibodies with the Watanabe and fat-fed rabbit. Arteriosclerosis 6: 601 – 13

Walfish PG, Gottesman IS, Shewchuk AB, Bain J, Hawe BS, Farid NR, 1983. Association of premature ovarian failure with HLA antigens. Tissue Antigens 21:168 – 69

Wilkinson JM, Wetterskog DL, Sogn JA, Kindt TJ, 1984. Cell surface glycoproteins of rabbit lymphocytes: characterization with monoclonal antibodies. Mol Immunol 21:95 – 103

Winchester RJ, Kunkel HG, 1979. The human Ia system. Adv Immunol 28: 221 – 92