HORMONAL REGULATION OF MACROPHAGE COLLAGENASE ACTIVITY

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Summary

Whereas peritoneal macrophages from nonpregnant guinea pigs were stimulated in vitro by endotoxin to produce collagenase on the second day of culture, those from pregnant guinea pigs were incapable of this response. However, if the cells from pregnant animals were preincubated for one day prior to endotoxin stimulation, collagenase activity could be detected. Injection of either estrogen or progesterone into guinea pigs at doses comparable to those found during pregnancy prior to removal of the peritoneal cells also inhibited the in vitro stimulation of collagenase production. The addition of these hormones in vitro revealed that at 5 x 10^{-6} M estrogen and progesterone inhibited 53% and 100% respectively of the collagenase activity. Addition of both hormones at a final concentration of 5 x 10^{-7} M of each inhibited 87% of the activity indicating a synergistic effect since this concentration of either hormone alone was ineffective.

INTRODUCTION

During pregnancy the inflammatory lesions associated with disorders such as rheumatoid arthritis (1) and lupus erythematosus (2) frequently improve. The mechanism by which this amelioration occurs is not known. However it is possible that the hormones which increase during gestation can regulate or inhibit the metabolic activities of the cell types responsible for the connective tissue alterations observed in these lesions. A predominant cell associated with chronic inflammatory lesions is the macrophage. We have demonstrated that macrophages activated either by endotoxin (3) or lymphokines (4) produce the enzyme collagenase which may be associated with the connective tissue destruction observed in these diseases. It was therefore of interest to determine whether pregnancy and specifically the elevation of hormone levels during pregnancy influenced the ability of macrophages to produce collagenase. Our findings indicate that estrogen and progesterone have an inhibitory effect on the production of collagenase by macrophages.

MATERIALS AND METHODS

Macrophages were obtained from female guinea pigs and cultured as previously described (3). Collagenase production by the macrophages was initiated by adding endotoxin (30 µg/ml) from Escherichia coli (055:B5 Difco Laboratories, Detroit, Mich.) to the culture. The media was harvested daily, dialyzed against several changes of distilled water, buffered to pH 7.5 with 30 M Tris and lyophilized. Collagenase activity was assayed by adding the lyophilized media products from 5×10^{-7} cells resuspended in 200 µl of 50 mM Tris-HCl (pH 7.5) plus 5 mM $CaCl_2$ to 300 µg of [^{14}C] glycine-labeled reconstituted collagen fibers (5) which had been obtained by acid extraction of guinea pig dermis and purified after the method of Kang et al (6). The extent to which proteases other than collagenase might degrade the collagen gel was estimated by adding trypsin (0.01%) to some of the collagen gels. After incubation at 35°C for 16 hr the insoluble collagen was removed by centrifugation and the solubilized labeled material present in the supernatant fluid was measured by liquid scintillation counting.

The effect of pregnancy or hormones on collagenase production by activated macrophages was evaluated by several experimental approaches. First, macrophages were obtained from pre- and post-partum guinea pigs. The pre-partum animals were injected intraperitoneally with 20 ml of sterile mineral oil (Drakeol, Pennsylvania Refining Co., Butler, Pa.) approximately 15 days prior to delivery and the peritoneal exudate cells harvested 4 days later. Post-partum animals were given intraperitoneal injections within 24 hr after delivery and the peritoneal exudate cells harvested 4 days later. Endotoxin was added to the media either on the first or second day of culture. The media from the first and second days after the addition of endotoxin were assayed for collagenase. Macrophages were also obtained from a group of guinea pigs that had received a series of hormonal injections subcutaneously every 12 hr for 8 days. The hormones injected were estradiol 17β benzoate (Shering Corp., Kenilworth, N.J., 1.0 µg per injection) and/or progesterone (Lilly, Indianapolis, Ind., 0.5 mg per injection.) in oil vehicle. These dosages are within the daily production levels of estrogen and progesterone by guinea pigs in late gestation (7). Mineral oil was injected intraperitoneally on the fifth day of hormone treatment and the peritoneal cells harvested 4 days later. The peritoneal exudate cells were incubated for 2 hrs and washed to remove nonadherent cells. Endotoxin was added on the first and second day of culture and media from each of the first 3 days of culture assayed for collagenase.

The macrophages obtained from a third group of animals were cultured with or without hormones for 24 hr. Media were removed and replaced with media containing hormones and endotoxin. Media were harvested on the first and second days after the addition of endotoxin and assayed for collagenase activity. The hormones (Calbiochem., La Jolla, Calif.) tested were estradiol-17β benzoate, and progesterone. The hormones were dissolved in absolute ethanol and added to the media at a final concentration of 200 µl per 100 ml of media.

RESULTS

Macrophages obtained from pre-partum guinea pigs did not produce collagenase when exposed to endotoxin during the first day of culture (Fig. 1). This was in contrast to macrophages from post-partum animals which released significant levels of collagenase following endotoxin

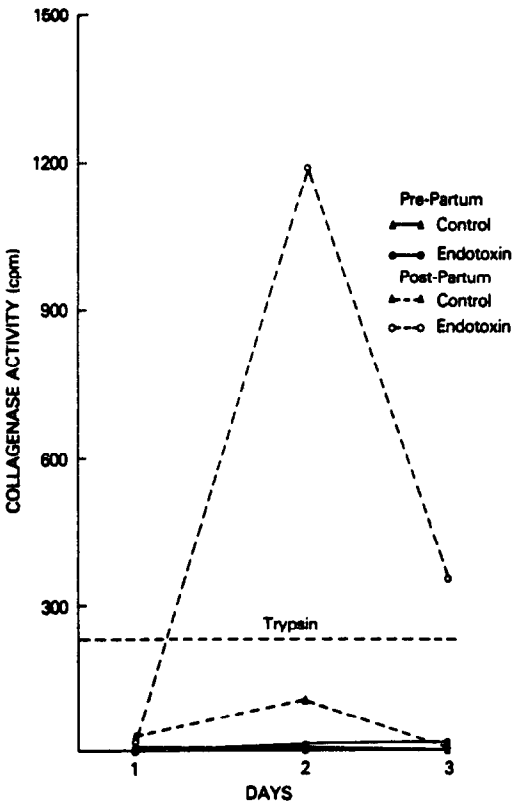

Figure 1. Comparison of collagenase activity of macrophages from pre-partum and post-partum guinea pigs. Peritoneal exudate cells were obtained from 9-10 day pre-partum and 5-6 day post-partum guinea pigs. The cells were cultured for 2 hr and than washed to remove the non-adherent cells. Endotoxin (30 µg/ml) was then added and the media removed daily for 3 days and assayed for collagenase (2110 cpm/300 µg of substrate).

stimulation. However, if macrophages from pre-partum guinea pigs were incubated for a day and then endotoxin added with fresh media, collagenase activity could be detected in the culture supernatants (Table 1). The ability of the macrophages from pregnant guinea pigs to recover enzyme production after a period of preincubation suggested a reversible influence of gestational hormones.

The possibility that hormones affect the macrophages *in vivo* to render them refractory to *in vitro* stimulation was evaluated by obtaining peritoneal exudate macrophages from nonpregnant female guinea pigs

TABLE I

RECOVERY OF MACROPHAGE COLLAGENASE PRODUCTION FROM HORMONAL CONTROL

PREGNANT GUINEA PIG MACROPHAGES[a]	COLLAGENASE ACTIVITY[c] (CPM [^{14}C] COLLAGEN SOLUBILIZED)	
ENDOTOXIN ADDED ON[b]	CULTURE AFTER ENDOTOXIN (DAYS)	
	1	2
DAY 1	15	44
DAY 2	4	997

a) Peritoneal exudate cells were acquired from 5-6 day pre-partum guinea pigs. After 2 hr of incubation the cultures were washed twice to remove the nonadherent cells.

b) Endotoxin (30μg) was added on the first or second day of culture.

c) Media was harvested on the first and second days after the addition of endotoxin and assayed for collagenase (2110 cpm/300μg of substrate).

TABLE 2

INFLUENCE OF IN VIVO HORMONE ADMINISTRATION ON MACROPHAGE COLLAGENASE PRODUCTION

PERITONEAL MACROPHAGES[a]	COLLAGENASE PRODUCTION[b] (CPM [^{14}C] COLLAGEN SOLUBILIZED)		
	DAY OF CULTURE		
	1	2	3
CONTROL	53	1993	2046
ESTROGEN	0	156	382
PROGESTERONE	14	35	571
ESTROGEN + PROGESTERONE	15	0	330

a) Peritoneal macrophages were acquired from control and hormonally treated animals. Hormones were injected every 12 hr for eight days. Mineral oil was injected intraperitoneally on the fifth day of hormonal injections and the peritoneal exudate cells harvested four days later.

b) The macrophages were cultured for 2 hr and washed to remove nonadherent cells. Endotoxin (30 μg/ml) was added to all cultures. Media were removed daily and collagenase activity was determined in each day's media (2236 cpm/300μg substrate).

TABLE 3

INFLUENCE OF IN VITRO HORMONE EXPOSURE ON MACROPHAGE COLLAGENASE PRODUCTION

HORMONE TREATMENT[a]	% INHIBITION OF MACROPHAGE COLLAGENASE[b]
ESTROGEN	
1×10^{-6}M	0
5×10^{-6}M	53
1×10^{-5}M	83
PROGESTERONE	
5×10^{-7}M	0
1×10^{-6}M	9
5×10^{-6}M	100
ESTROGEN + PROGESTERONE	
1×10^{-7}M + 1×10^{-7}M	0
5×10^{-7}M + 5×10^{-7}M	87
1×10^{-6}M + 1×10^{-6}M	100

a) Representative experiment in which peritoneal exudate cells were placed in culture and hormones added at the indicated final concentrations. Twenty-four hr later the media were removed and fresh media, endotoxin (30µg/ml) and hormones were added to the cultures. Control cultures contained endotoxin but no hormones.

b) Media were removed daily and the media harvested on the second day after the addition of endotoxin used to determine collagenase activity.

injected with estrogen or progesterone or both hormones. Addition of endotoxin to these macrophages revealed that the macrophages from control animals (injected with vehicle only) produced significant amounts of collagenase while the cells from animals administered estrogen or progesterone or both did not produce detectable collagenase within 48 hr after exposure to endotoxin (Table 2). However, collagenolytic activity could be detected in the day 3 media, although at lower levels than that in control cultures. Since macrophages from hormone injected animals were inhibited in their endotoxin induced production of collagenase it was of interest to determine the effects of these hormones administered directly in vitro. Hormones were added to the macrophage cultures a day prior to endotoxin addition. This re-

sulted in more effective inhibition of collagenase than simultaneous addition of hormones and endotoxin. The data revealed that estrogen inhibits 53% of the activity at 5×10^{-6} M, while progesterone inhibited 100% of the collagenolytic activity at this concentration (Table 3). When both hormones were added at a final concentration of 5×10^{-7} M each, a concentration at which neither estrogen nor progesterone was inhibitory, 87% of the activity was abolished demonstrating a synergistic effect.

DISCUSSION

The regulation of tissue collagenase by hormones has been demonstrated in several laboratories. Jeffrey et al (8) found that at pharmacological doses progesterone (5×10^{-5}M) or its analogue, 6 α-methyl, 17 α-acetoxy progesterone (1×10^{-6}M) when added to the media of cultured uterine explants inhibited collagenase activity. In their studies estradiol (1×10^{-5}M) alone did not inhibit this enzyme activity but did seem to potentiate the effect of progesterone. In contrast, Woessner (9) and Ryan and Woessner (10) were able to inhibit collagenase activity in the post-partum rat uterus with injections of estrogen.

The main cell type(s) involved in the production of collagenase in these hormonally controlled systems is not known, although macrophages have been demonstrated to increase significantly in the uterus during resorption and contain vacuoles filled with collagen (11). Thus, it was significant that macrophages obtained from pregnant guinea pigs during a period when hormone levels are high were refractory in their ability to produce significant levels of collagenase when stimulated by endotoxin. That the inhibition of collagenase activity could be attributed to the influence of gestational hormones was further documented by our findings in nonpregnant guinea pigs injected with these hormones. When the animals were injected with either estrogen or progesterone at concentrations comparable to those found during pregnancy macrophage enzyme

activity was significantly inhibited. These findings are consistent with those of Bodel et al (12) in which the metabolic functions of leukocytes have shown to be affected during pregnancy. They have shown that leukocytes from pregnant women have reduced production of CO_2 after phagocytic activity.

It was evident that macrophages obtained during pregnancy do not produce detectable collagenase activity in response to endotoxin during the first day of culture. However, this inhibitory effect was reversible if the macrophages from pregnant animals were allowed to incubate for 24 hr prior to the addition of endotoxin. That this effect is related to gestational hormones is indicated by the lack of collagenase activity associated with endotoxin-treated macrophages from guinea pigs injected with physiological doses of estrogen or progesterone, and additionally by the in vitro inhibitory activity of these hormones on the macrophage system. The in vitro addition of these hormones to macrophages for 24 hr prior to the addition of endotoxin revealed progesterone to be the more potent inhibitor of collagenase activity. However, under the conditions established in culture neither hormone alone could inhibit collagenase activity at physiological doses. This lack of effect may be related to the short period of time the macrophages were exposed to hormones since the inhibitory effect was found to be related to the length of time the cells were incubated with the hormones. In this regard the length of exposure to hormones would be much greater in the in vivo studies which may explain why physiological doses could inhibit the activation of macrophages leading to collagenase production. Moreover, when estrogen and progesterone were added together at a concentration of 5×10^{-7}M of each, a level at which neither hormone inhibited, 87% of the collagenase activity was inhibited. It has been noted that progesterone uptake (13) is increased in the guinea pig uterus by estrogen treatment, presumably by an increase in receptor sites (14). Thus,

it may be that both hormones are required for maximal inhibition of macrophage enzyme activity.

From these findings one may speculate that during gestation the increased levels of hormones may have a regulatory influence on macrophage function. Inhibition of macrophage collagenase activity by these hormones could account for the diminished inflammatory response seen in certain connective tissue lesions during pregnancy.

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