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Cell-Cell Interactions in the Testis of Adjuvant-Induced Arthritic Rat

ANGELA L. ASIRVATHAM AND BRENT C. BRUOT

From the Department of Biological Sciences, Kent State University, Kent, Ohio.

ABSTRACT: Rats with adjuvant-induced arthritis (AA) have low levels of serum testosterone, and production of testosterone reportedly is influenced by macrophage secretory products. This study was undertaken to understand the mechanism mediating this hypoandrogenism. Testicular macrophages from AA and nonarthritic (NA)rats were cultured, and conditioned media was added to testicular interstitial cells and Percoll-purified cells from NA rats. Testosterone production by interstitial cells stimulated with luteinizing hormone (LH) and incubated with adjuvant-induced arthritic macrophage conditioned medium (AAMCM) was significantly lower than in cells incubated with nonarthritic macrophage conditioned medium (NAMCM). However, there was no difference in testosterone production by Percoll-purified Leydig cells and those stimulated with LH when incubated with AAMCM or NAMCM. To determine whether an intermediary cell type was involved in mediating inhibition of testosterone production, AAMCM and NAMCM were added to a reconstituted preparation of testicular interstitial cells. Addition of AAMCM restored the inhibitory effect, suggesting that arthritic hypoandrogenism is mediated by cell-cell interaction. These results suggest that a factor produced by macrophages from AA rats appears to mediate testosterone production by acting in conjunction with other cells in the testicular interstitium.

Key words: Arthritis, hypoandrogenism, Leydig cells testosterone, testicular macrophages.

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During the last decade, several studies have explored the relationship between the immune system and endocrine function of the testis. In the male gonad, testosterone production appears to be influenced by products secreted by macrophages (Yee and Hutson, 1985). Testicular macrophages secrete a variety of cytokines, and many studies have been conducted to examine the effects of cytokines on cultured Leydig cell testosterone production (Guo, 1990; Hales, 1992; Li, 1995). Clinical studies conducted by Spector et al (1989) found that men with rheumatoid arthritis (RA) have significantly lower levels of testosterone than healthy controls. However, very little is known about the effect of macrophage activation on testicular function.

The adjuvant-induced arthritis (AA) rat model is widely used in the study of RA and has been used to study the effects of macrophage activation on testicular function. Male Lewis rats with AA are known to have reduced levels of serum testosterone, similar to men with RA (Clemens and Bruot, 1989). Preliminary studies of cultured testicular macrophages from AA rats were reported to secrete a factor that could inhibit testosterone production by nonarthritic (NA) cultured testicular interstitial cells (Bruot and Clemens, 1992).

Correspondence to: Dr Angela Asirvatham, Veterans Affairs Medical Center, R&D-8, 3710 SW U.S. Veterans Hospital Road, Portland, OR 97201.

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The purpose of this study was to extend the initial observations on testicular steroidogenesis.

Materials and Methods

Induction of Arthritis

Male inbred Lewis rats (aged 55–60 days; Charles Rivers Laboratory, Wilmington, Mass) were assigned to NA (control) and AA groups. The rats were housed in microisolation cages under controlled lighting (12 hours light:12 hours dark) in a temperature controlled room (21 ± 2°C). All procedures were approved by the Kent State University animal care and use committee.

Arthritis was induced by injecting heat-killed *Mycobacterium butyricum* that had been ground into a fine powder and suspended in Freunds incomplete adjuvant (DIFCO, Detroit, Mich) to a concentration of 10 mg/mL (Billingham and Davies, 1982). Rats were anesthetized, and a 0.1-mL aliquot of the bacterial solution was injected intradermally at the base of the tail before the injection. Animals were assessed for arthritis on day 21 by determining body weight and hind paw volume (Bruot and Clemens, 1992). Animals were killed by decapitation, and trunk blood was collected for hormone analysis.

In Vitro Studies

Testicular Macrophage Culture—Testes were removed and testicular macrophages were cultured for 24 hours; macrophages were isolated as described in Bruot and Clemens (1992) and Hutson and Doris (1996). Testes were decapsulated and dispersed in Dulbecco phosphate buffered saline (DPBS pH 7.4; Sigma Chemical Company, St Louis, Mo) containing 0.025% collagenase (Worthington Biochemicals, Freehold, NJ) by shak-

ing at the rate of 140 cycles/min for 20 minutes. After dispersion, seminiferous tubules were removed by filtering them through a fine organza mesh. The supernatant was collected and the filtrate was centrifuged at 500 × *g* in a Sorvall (Newton, Conn) centrifuge with an H1000B rotor at 4°C for 10 minutes.

Meanwhile, minimum essential medium (MEM with Earle salts and L-glutamine pH 7.2; Sigma) supplemented with 0.1% bovine serum albumin (BSA; Sigma), 100 U penicillin (Sigma), 100 μg streptomycin (Sigma), and 5 μg insulin was added to Gibco 100-mm diameter Petri dishes and allowed to equilibrate in the incubator so that the medium would have the optimum pH and temperature. The cell suspension, consisting of 2 million cells/mL, was suspended in the equilibrated medium and plated into Gibco 100-mm-diameter Petri dishes. The cells were allowed to plate for 3 minutes in a CO_2 incubator (95% air and 5% CO_2) at 34°C. Approximately 20%–25% of the cells (0.4–0.5 million) were macrophages. The unattached cells, consisting of Leydig cells, fibroblasts, and others were removed by gentle washing and swirling the plate 20 times. The washing procedure was conducted three times. Almost all nonadherent cells were removed with this process.

Fresh medium was added to the plated cells. The adherent macrophages were cultured for 24 hours, and the conditioned medium was collected and used in subsequent experiments. The pH of the medium was maintained between 7 and 7.4. DNA assays were performed after the macrophage cultures were terminated to confirm that cell concentrations in both AA and NA cultures were similar (11 μg and 11.3 μg for AA and NA macrophage cultures, respectively). Cells were 90%–96% viable in both AA and NA cell populations.

In an independent experiment it was determined that cells harvested using this procedure were revealed to be 85% positive for ED-2, a macrophage-specific marker (Dijkstra et al, 1985; Raburn et al, 1993), and less than 4% stained positively for the steroidogenic enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) isomerase.

To control for an effect of incubation on MEM in this experiment, a series of plates were incubated without macrophages for 24 hours, and the control conditioned medium (CM) was collected and stored frozen. Medium collected from AA, NA, and CM cultures were treated in a similar manner and stored at −20°C. AA, NA, and CM media used from freshly isolated cells or stored at −20°C had similar abilities to modulate testosterone production.

Testicular Interstitial Cell Culture—Adult Sprague-Dawley rats (at least 60 days of age) were killed by CO_2 asphyxiation and testes were removed. Interstitial cells were prepared as described by Dufau et al (1974) and Bruot and Clemens (1992). The testes were decapsulated and dispersed with 0.025% collagenase in DPBS in a 50-mL polyethylene tube. The tube was incubated in a 37°C water bath and shaken at 150 cycles/min. After 15 minutes the seminiferous tubules were removed by filtering through an organza mesh. The filtrate was centrifuged at 300 × *g* for 15 minutes to pellet the dispersed cells. The pelleted cells, consisting of 2 million cells/mL, were suspended in MEM and plated in 100-mm Petri dishes for 3 minutes in a CO_2 incubator to remove macrophages. The unattached cells were removed by gentle rinsing.

The washes obtained after macrophage adherence were collected and centrifuged at 300 × *g* for 15 minutes at 4°C. The pelleted cells were resuspended in Dulbecco modified Eagle medium (DMEM)/F12 (with L-glutamine and 15 mM Hepes (pH 7.2; Sigma) supplemented with 0.1% BSA, 100 U penicillin, 100 U streptomycin (Sigma), 5 μg insulin (Sigma), 1 mg bovine transferrin (Sigma), and plated at a concentration of 3×10^5 cells/mL in a multiwell plate (Falcon). Approximately 15%–20% of the cells isolated using this procedure are positive for 3β-HSD enzyme. Testicular interstitial cells were incubated with 100 μL (10%), 200 μL (20%), and 500 μL (50%) of adjuvant-induced arthritic macrophage conditioned medium (AAMCM), or nonarthritic macrophage conditioned medium (NAMCM), or CM in a total volume of 1 mL. In some experiments, AAMCM, NAMCM, and CM were cultured with 1 ng/mL LH (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) for 24 hours in a CO_2 incubator. The medium was collected at the end of 24 hours, and stored frozen at −20°C until analyzed for testosterone.

Isolation of Leydig Cells and Non-Leydig Cells—Leydig cells were isolated as described by Browning et al (1981) and Bruot and Clemens (1992). After macrophages were isolated the nonadherent cells were washed by a gentle jet stream. The washes after macrophage adherence were centrifuged, and the pellet was resuspended in 2 mL of DMEM/F12. The 2-mL cell suspension was loaded onto a self-generating continuous Percoll gradient made of 16.5 mL isosmotic Percoll (15 mL Percoll and 1.5 mL 10× concentrated M199) and 11.5 mL M199–0.1% BSA (M199 with Earle balanced salts and L-glutamine pH 7.4, Sigma; supplemented with 100 U penicillin, 100 U streptomycin, 5 μg insulin, and 1 mg bovine transferrin). The resultant gradient was centrifuged at 13 000 × *g* in a Sorvall RC5C centrifuge with the SS-34 rotor for 1 hour, and the upper fraction was removed. Fraction numbers 25–30 were removed and split into 5 parts. Each part was washed with DPBS and centrifuged at 300 × *g* for 20 minutes. The supernatant was removed and the pellets were suspended in DMEM/F-12 and plated at a concentration of 1×10^5 cells/mL. The remaining fractions consisting of non-Leydig cells were used for subsequent experiments.

Leydig cells were incubated with 100 μL (10%), 200 μL (20%), and 500 μL (50%) of AAMCM, NAMCM, or CM in a total volume of 1 mL. In some experiments, AAMCM, NAMCM, or CM were cultured with 1 ng/mL LH for 24 hours in a CO_2 incubator. The medium was collected at the end of 24 hours, and stored frozen at −20°C until analyzed for testosterone.

Leydig cells isolated in this manner are approximately 80%–85% positive for 3β-HSD enzyme. The percentage of macrophages in the Leydig cell preparation was not determined. However, Laws et al (1985), using a similar technique, found that 3% of the cells in the Leydig cell fraction were macrophages. This fraction was taken from a similar position on the Percoll gradient and contained a percentage of Leydig cells similar to the preparations used in this study. In an independent experiment (data not shown) there was no significant difference in the distribution of 3β-HSD positive cells in gradients prepared from testes of arthritic and nonarthritic animals.

Reconstitution of Interstitial Preparation

Interstitial cells from Sprague-Dawley rats were isolated as described above. After decapsulation of testes, the testicular dispersion was filtered and the filtrate was centrifuged at 300 × *g* for 15 minutes at 4°C. The pelleted cells were suspended in M199–0.1% BSA, and 2 mL of the suspension were loaded on a self-generating continuous Percoll gradient to isolate Leydig cells (Browning et al, 1981; Bruot and Clemens, 1992) as mentioned earlier. The remaining 1 mL of the suspension was used later for culture. The gradient was centrifuged at 13 000 × *g* for 1 hour at 4°C. Fractions 24–27 containing Leydig cells were pooled together and washed in DPBS. Fractions 1–23 and Fractions 28, 29, and 30, consisting of cells other than Leydig cells, were combined and also washed with DPBS.

The various cell fractions were stained for 3β-HSD enzyme to ascertain the percentage of Leydig cells. Approximately 16%–20% of the interstitial cell cultures and 80%–85% of the Leydig cell culture exhibited positive staining for 3β-HSD enzyme. Cultures consisting of non-Leydig cells stained 6%–8% positive for 3β-HSD enzyme, and cultures containing Leydig and non-Leydig cells were approximately 17%–20% positive for 3β-HSD.

Design of Culture

Aliquots of AAMCM, NAMCM, or CM at 50% concentrations were incubated with the following group of cells: testicular interstitial cells were plated at a concentration of 3×10^5 cells/mL, Leydig cells (Percoll gradient fraction 24–27) at 1×10^5 cells/mL, non-Leydig cells (fraction 1–23, 28, 29, and 30) at 1×10^5 cells/mL, and Leydig cells (fraction 24–27) with non-Leydig cells (fractions 1–23, 28, 29, and 30) at 3×10^5 cells/mL. The cells were cultured for 24 hours at 34°C in a CO_2 incubator. Medium was collected at the end of 24 hours and stored frozen at −20°C until analyzed for testosterone.

Hormone Analysis

Testosterone was analyzed by radioimmunoassay as reported in Lee and Bruot (1986). The intraassay and interassay coefficients of variability were less than 10%.

Statistical Analysis

Statistical analysis was performed by analysis of variance and post hoc tests using SPSS version 9.0 (SPSS Inc, Chicago, Ill) as the statistical package. Comparisons between means were made with the Newman-Keul, Duncan, or least square differences tests. In analysis involving more than one factor, interaction effects were included in the model. Due to differences in basal secretion of testosterone between experiments by both Leydig and interstitial cell cultures, all values are graphically represented as a percent of CM. However, the pattern of testosterone production by cultures incubated with various treatments in each individual experiment were consistent. All percentage values are represented as the mean ± SEM of the number of culture experiments.

Testosterone secretion was corrected for the number of 3β-HSD positive Leydig cells and calculated as ng/mL/3×10^5 cells for interstitial cells and ng/mL/10^5 cells for Leydig cell cultures.

Table. *Body weights, paw volumes, and serum testosterone in arthritic and nonarthritic rats**

Parameter	Arthritic rats (Means ± SEM, n = 34)	Nonarthritic rats (Means ± SEM, n = 34)
Body weight (g)	243 ± 5.1[a]	312 ± 4.4[b]
Paw volume (mL)	1.67 ± 0.01[a]	1.08 ± 0.02[b]
Serum testosterone (ng/mL)	3.43 ± 0.6[a]	6.09 ± 0.43[b]

* Different letters denote a significant difference. Means of body weights are significant at $P < .001$, means of paw volumes are significant at $P < .005$, and means of serum testosterone are significant at $P < .01$.

Results

Effect of Arthritis

Body weights of rats with arthritis were significantly lower than nonarthritic rats. Serum testosterone levels in AA rats were significantly lower than NA rats. Paw volumes of AA animals were significantly higher than NA rats (Table). Analysis of paw volume data indicates that animals with paw volumes greater than 1.5 mL had lower levels of testosterone compared with animals with paw volumes less than 1.5 mL. A significant negative correlation ($r = -.434$ at $P = .004$) between the degree of paw swelling and levels of serum testosterone was observed.

Effect of AAMCM on Basal and LH-Stimulated Testosterone Production by Interstitial Cells

The effect of AAMCM on testosterone production by interstitial cells was evaluated in this experiment. Addition of AAMCM to interstitial cultures resulted in a significant dose-dependent decrease in testosterone production compared with interstitial cells incubated with NAMCM. Interstitial cells incubated with NAMCM produced 106% ± 9%, 97% ± 5%, and 110 ± 5% at 10%, 20%, and 50%, of CM, respectively. In comparison, interstitial cells incubated with 10%, 20%, and 50% concentrations of AAMCM secreted 100% ± 5%, 92% ± 4%, and 78% ± 6% of CM, respectively. The addition of 50% AAMCM to interstitial cells resulted in a 32% reduction of testosterone production compared with cultures incubated with NAMCM. This reduction was significantly different from other treatments (Figure 1A).

A similar decrease in testosterone secretion was observed when interstitial cells were cultured with 1 ng/mL LH (Figure 1B). Interstitial cells cultured with 1 ng/mL LH and NAMCM secreted testosterone levels of 98% ± 4%, 108% ± 5%, and 107% ± 7% at, respectively, 10%, 20%, and 50% concentrations of medium. Addition of AAMCM with 1 ng/mL LH to interstitial cells resulted in testosterone production of 100% ± 7%, 93% ± 3%, and 76% ± 4% at, respectively, 10%, 20%, and 50% concentrations. Incubation of 50% AAMCM with 1 ng/mL LH significantly reduced testosterone production by 31% compared to cultures incubated with NAMCM. This decrease was significantly different from that of other treatments.

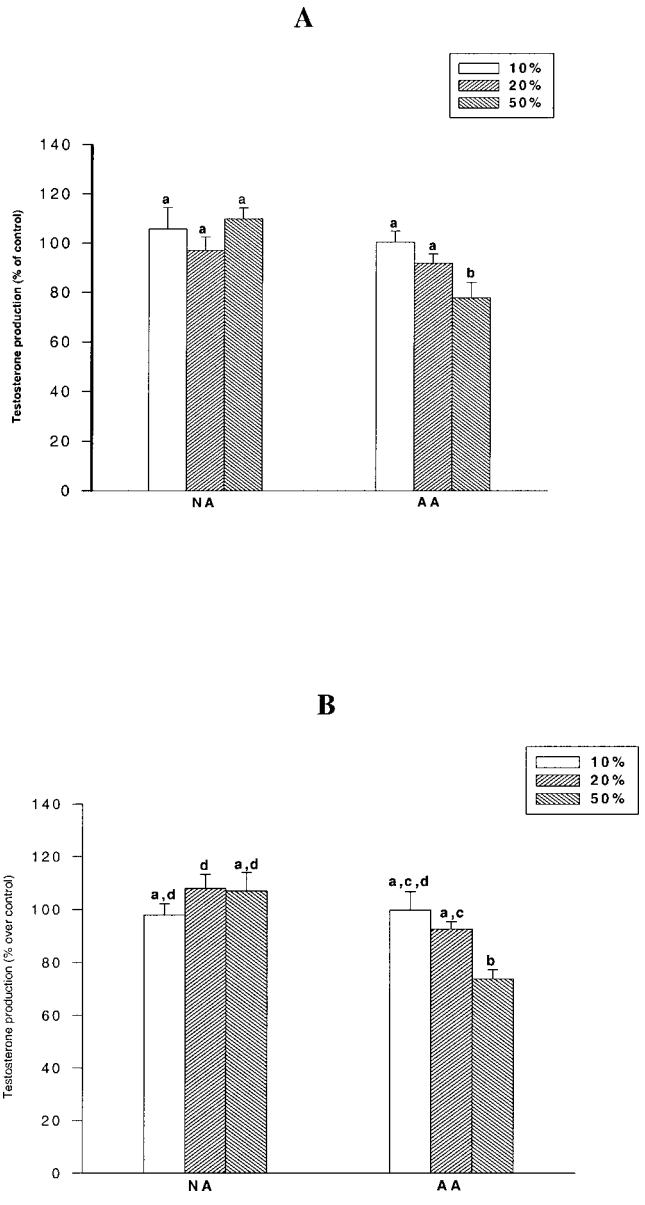


Figure 1. **(A)** Interstitial cell testosterone secretion in response to conditioned media. Cells were incubated with 100 (10%), 200 (20%), and 500 μL (50%) of AAMCM, NAMCM, or CM. Testosterone production is expressed as a percentage of CM. Basal secretion of testosterone by interstitial cells incubated with NAMCM at 10%, 20%, and 50% were 2.18, 2.26, and 2.14 ng/mL/3 $\times$ 10^5 cells, respectively. Interstitial cells incubated with AAMCM at 10%, 20%, and 50% secreted 1.83, 1.72, and 1.45 ng/mL/3 $\times$ 10^5 cells, respectively. Interstitial cultures incubated with CM secreted 1.97, 2.08, and 1.8 ng/mL/3 $\times$ 10^5 cells of testosterone at 10%, 20%, and 50% concentrations of media. Test results are expressed as the mean $\pm$ SEM of 7 experiments for basal secretion of testosterone. Different letters denote significance differences in testosterone production ($P < .05$). **(B)** Interstitial cells were also incubated with 1 ng/mL LH and 100 (10%), 200 (20%), and 500 μL (50%) of AAMCM, NAMCM, or CM. Testosterone production is expressed as a percentage of control. Testosterone production of cells incubated with 1 ng/mL LH and 10%, 20%, and 50% NAMCM were 12.5, 12.7, and 12.9 ng/mL/3 $\times$ 10^5 cells. Interstitial cells treated with AAMCM at 10%, 20%, and 50% secreted 12.54, 11.22, and 9.75 ng/mL/3 $\times$ 10^5 cells. Cultures incubated with 10%, 20%, and 50% of CM secreted 12.97, 12.2, and 12.5 ng/mL/3 $\times$ 10^5 cells. Results for cultures incubated with 1 ng/mL LH are expressed as the mean $\pm$ SEM of 6 experiments. Different letters denote significance in testosterone production ($P < .05$).

Effect of AAMCM on Basal and LH-Stimulated Cultures by Leydig Cells

The effect of AAMCM on testosterone production by Percoll-purified Leydig cells was investigated. AAMCM had no effect on testosterone production by Leydig cells compared with cultures incubated with NAMCM (Figure 2A). Incubation of Leydig cells with NAMCM secreted 114% $\pm$ 13%, 113% $\pm$ 12%, and 129% $\pm$ 10% of CM at 10%, 20%, and 50% concentrations of medium, respectively. However, cells incubated with AAMCM resulted in a nonsignificant, dose-dependent increase in testosterone production (108% $\pm$ 14%, 114% $\pm$ 14%, and 123% $\pm$ 18% at, respectively, 10%, 20%, and 50% concentrations of medium).

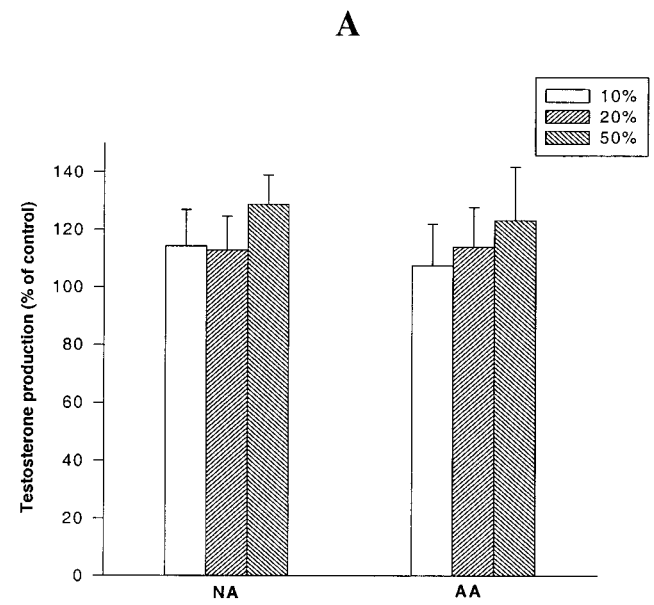
Testosterone production by Leydig cell cultures incubated with AAMCM and 1 ng/mL LH did not differ from the cultures incubated with NAMCM and 1 ng/mL LH at 10%, 20%, or 50% concentrations of media (Figure 2B). Addition of NAMCM with 1 ng/mL LH to Leydig cells at 10%, 20%, and 50% concentrations resulted in a dose-dependent increase in testosterone production of 98% $\pm$ 9%, 115% $\pm$ 10%, and 117% $\pm$ 8%, respectively, although this was not significant. Leydig cells incubated with 10%, 20%, and 50% concentrations of AAMCM and 1 ng/mL LH secreted, respectively, 115% $\pm$ 12%, 129% $\pm$ 17%, and 115% $\pm$ 12% of testosterone.

Effect of AAMCM on Reconstituted Preparation of the Testis

In this experiment, the effect of AAMCM and NAMCM on a reconstituted preparation of testicular cells from NA rats was examined. Addition of 50% AAMCM to interstitial cells resulted in a significant decrease in testosterone production (67% $\pm$ 13%) compared with interstitial cells incubated with NAMCM (102% $\pm$ 7%). The inhibitory effect was lost when AAMCM was added to cultured, Percoll-purified Leydig cells. Testosterone production by Leydig cells incubated with AAMCM and NAMCM was 104% $\pm$ 8% and 106% $\pm$ 7%, respectively. Similarly, incubation of AAMCM and NAMCM with non-Leydig cells fractions had no effect on testosterone production (120% $\pm$ 17% and 126% $\pm$ 12%, respectively). However, the inhibitory effect was restored when AAMCM was added to reconstituted cells (Leydig + non-Leydig cells; 85% $\pm$ 5%) compared with cells incubated with NAMCM (123% $\pm$ 13%; Figure 3).

Discussion

Results from this experiment confirm previous studies (Clemens and Bruot, 1989) that adjuvant-induced arthritic animals secrete less testosterone compared with nonarthritic rats. Animals with paw volumes higher than 1.5 mL had lower levels of testosterone. Although arthritic animals with paw volumes lower than 1.5 mL had a mild to moderate manifestation of arthritis, serum testosterone



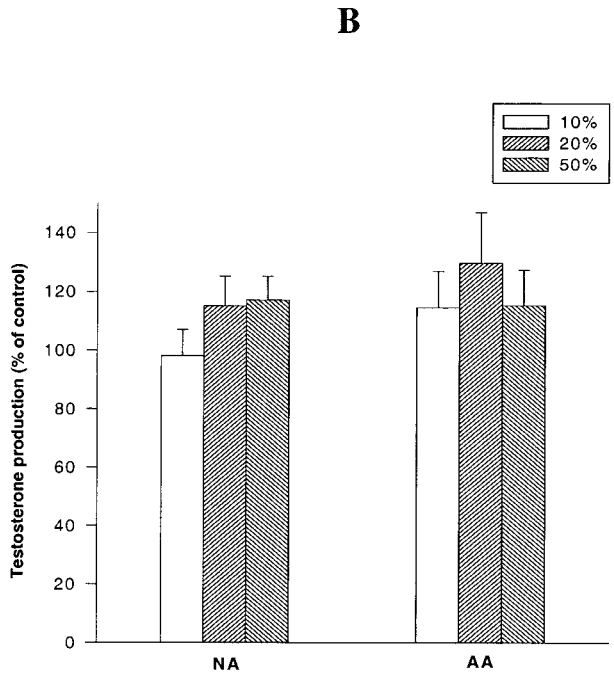


Figure 2. **(A)** Purified Leydig cell response to conditioned media. Leydig cells were incubated with 100 (10%), 200 (20%), and 500 μL (50%) of AAMCM, NAMCM, or CM. Testosterone production is expressed as a percentage of the control. The results are expressed as the mean ± SEM of 6 experiments. Testosterone secretion by Leydig cells incubated with 10%, 20%, and 50% NAMCM were 9.79, 9.78, and 10.41 ng/mL/1 × 10^5 cells, respectively. Leydig cells incubated with AAMCM secreted 9.18, 10.08, and 10.17 ng/mL/1 × 10^5 cells at 10%, 20%, and 50% concentrations, respectively. Leydig cells incubated with CM secreted 7.97, 8.48, and 7.9 ng/mL/1 × 10^5 cells at 10%, 20%, and 50% concentrations, respectively. **(B)** Leydig cells were also incubated with 1 ng/mL LH and 100 (10%), 200 (20%), and 500 μL (50%) of AAMCM, NAMCM, or CM. Testosterone production is expressed as a percentage of the control. Leydig cells treated with NAMCM at 10%, 20%, and 50% secreted 56.4, 55.5, and 56.9 ng/mL/1 × 10^5 cells, respectively. Testosterone secretion by Leydig cells incubated with 10%, 20%, and 50% AAMCM were 62.98, 62.77, and 57.7 ng/mL/1 × 10^5 cells, respectively. Leydig cells cultured with 10%, 20%, and 50% CM secreted 56, 51, and 49.1 ng/mL/1 × 10^5 cells. The results are expressed as the mean ± SEM of 6 experiments.

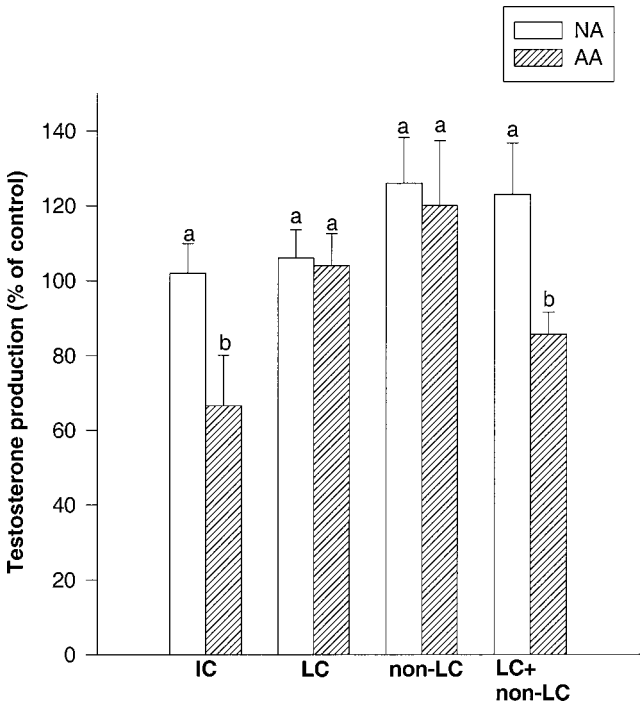


Figure 3. Effect of CM on a reconstituted preparation of testicular interstitial cells. Interstitial cells (IC), purified Leydig cells (LC fractions 24–27), non-Leydig cell fractions (non-LC consisting of cells other than Leydig cells; fractions 1–23, 28, 29, and 30), and Leydig cells with non-Leydig cells (LC + non-LC; fractions 24–27 + fractions 1–23, 28, 29, and 30) were incubated with an aliquot (50%) of AAMCM, NAMCM, and CM. Testosterone (ng/10^5 Leydig cells) secretion is expressed as a percentage of the control. Basal testosterone secretions by cultures incubated with NAMCM were 2.37, 9.36, 1.93, and 0.67 ng/mL for interstitial cells, LC, non-LC, and LC + non-LC, respectively. Cultures incubated with AAMCM secreted 1.22, 9.13, 1.78, and 0.47 ng/mL for interstitial cells, LC, and LC + non-LC. Cultures treated with CM secreted 2.27, 9.5, 1.57, and 0.52 ng/mL for interstitial cells, LC, and LC + non-LC, respectively. The results are expressed as the mean ± SEM of 4 experiments. Different letters denote a significant difference in testosterone production ($P < .05$).

in those animals was not significantly different from that in nonarthritic animals. These results suggest that a progressive decrease in serum levels of testosterone exacerbates the symptoms of arthritis, and that severity of arthritic symptoms are inversely related to serum levels of testosterone.

Addition of AAMCM to testicular interstitial cells resulted in a significant reduction in testosterone produc-

tion. Results from this study, in addition to previous reports by Bruot and Clemens (1992), clearly suggest that arthritic testicular macrophages secrete a modulator that reduces testosterone production by interstitial cells of nonarthritic animals. The inhibitory effect was also observed in LH-stimulated cultures, which suggests that the modulator is independent of LH.

However, addition of AAMCM had no effect on basal or LH-stimulated testosterone production by Percoll-purified Leydig cells. In fact, it was found that addition of AAMCM stimulated basal testosterone production by purified Leydig cells, although the increase was not significant. This increase in testosterone production agrees with previous reports that macrophage-conditioned medium stimulates testosterone production in normal animals (Yee and Hutson, 1985). Instead, the loss of AAMCM's ability to inhibit testosterone production raises the possibility that another type of cell present in the interstitial preparation may have functioned as an intermediary with macrophages in causing the inhibitory effect.

To examine this possibility, AAMCM was added to a reconstituted preparation of interstitial cells, which resulted in an inhibition of testosterone production. Restoration of the inhibitory effect in the reconstituted preparation clearly implies that testicular macrophages act in conjunction with other cells in the interstitial preparation to reduce testosterone production. Based on these results, it is clear that the arthritic testicular macrophage–Leydig cell interaction under in vitro conditions is not direct, but it involves interaction with other cells. It is also possible that interstitial cell cultures may contain a minimal amount of germ cells and Sertoli cells from the seminiferous tubules during testicular dispersion, which may play a marginal role in modulating the testosterone secretion by Leydig cells. However, in vivo, because the testicular interstitium contains fibroblasts, T lymphocytes, mast cells, peritubular myoid cells (Skinner, 1991; Hedger, 1997), and other cells in addition to macrophages and Leydig cells, it may be possible that a complex cell-cell interaction mediates this reduction in testosterone production in rats with arthritis.

Inhibition of testosterone secretion mediated by arthritic macrophages on interstitial cells may be attributed to various reasons. It is possible to speculate that the factor secreted by arthritic macrophages may be toxic in nature, thereby inducing cell death and resulting in a reduction in steroid secretion. However, the macrophage cultures were not visibly affected in terms of viability or morphology after 24 hours of culture. Because macrophage cultures were not confluent and were incubated for only 24 hours, depletion of nutrients in the media, which results in a reduction of testosterone secretion, is unlikely. Furthermore, the numbers of macrophages in both arthritic and nonarthritic CM were similar, as confirmed by DNA assays. The conditioned media from AA, NA, and CM were collected, stored, and treated identically. However, the inhibitory effect on steroid secretion was restricted to the arthritic macrophage CM only. The nature of the factor secreted by the arthritic macrophages is unknown.

Macrophages secrete a variety of products such as cytokines (Kern, 1995a), prostaglandins (Kern, 1995b), and nitric oxide (Pomerantz and Pitelka, 1998). Cytokines such as tumor necrosis factor-α, interleukin (IL)-1, IL-2, and interferon-γ have been found to inhibit gonadotropin or cAMP-stimulated steroidogenesis in Leydig cells at the level of expression of different steroidogenic enzyme genes (Calkins, 1988; Meikle,1992; Xiong and Hales, 1993). Macrophage-derived nitric oxide has been reported to inhibit androgen production by acting directly on Leydig cells via inhibition of the P450 steroidogenic enzymes (Del Punta et al, 1996; Pomerantz et al, 1998). Peroxide and other superoxide anions have also been reported to inhibit steroidogenesis (Behrman and Preston, 1978).

In conditions such as adjuvant-induced arthritis, testicular macrophages may become activated and secrete other inflammatory mediators in addition to cytokines and prostaglandins. Also, arthritic macrophages may be secreting a combination of secretory products that may mediate the androgen inhibition in the appropriate concentration. It is possible that the concentration of secretory products may be altered in pathological conditions such as AA in order to mediate arthritic hypoandrogenism.

Collectively, these results suggest that the modulator secreted by arthritic testicular macrophages appears to mediate hypoandrogenism by interacting with Leydig cells and other cells in the testicular interstitium. Experiments to identify the nature of the arthritic macrophage secretory product are currently in progress.

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