

Cooperativity between Sertoli Cells and Testicular Peritubular Cells in the Production and Deposition of Extracellular Matrix Components

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ABSTRACT We examined the synthesis and deposition of extracellular matrix (ECM) components in cultures of Sertoli cells and testicular peritubular cells maintained alone or in contact with each other. Levels of soluble ECM components produced by populations of isolated Sertoli cells and testicular peritubular cells were determined quantitatively by competitive enzyme-linked immunosorbent assays, using antibodies shown to react specifically with Type I collagen, Type IV collagen, laminin, or fibronectin. Peritubular cells in monoculture released into the medium fibronectin (432 to 560 ng/ μ g cell DNA per 48 h), Type I collagen (223 to 276 ng/ μ g cell DNA per 48 h), and Type IV collagen (350 to 436 ng/ μ g cell DNA per 48 h) during the initial six days of culture in serum-free medium. In contrast, Sertoli cells in monoculture released into the medium Type IV collagen (322 to 419 ng/ μ g cell DNA per 48 h) but did not form detectable amounts of Type I collagen or fibronectin during the initial six days of culture. Neither cell type produced detectable quantities of soluble laminin. Immunocytochemical localization investigations demonstrated that peritubular cells in monoculture were positive for fibronectin, Type I collagen, and Type IV collagen but negative for laminin. In all monocultures most of the ECM components were intracellular, with scant deposition as extracellular fibrils. Sertoli cells were positive immunocytochemically for Type IV collagen and laminin but negative for fibronectin and Type I collagen. Co-cultures of peritubular cells and Sertoli cells resulted in interactions that quantitatively altered levels of soluble ECM components present in the medium. This was correlated with an increased deposition of ECM components in extracellular fibrils. The data presented here we interpret to indicate that the two cell types in co-culture act cooperatively in the formation and deposition of ECM components. Results are discussed with respect to the nature of interactions between mesenchymal peritubular cell precursors and adjacent epithelial Sertoli cell precursors in the formation of the basal lamina of the seminiferous tubule.

In the intact testis, Sertoli cells and peritubular cells are adjacent to each other in the boundary tissue of the seminiferous tubule, separated by a basal lamina (1), with which only a relatively small percentage of the Sertoli cell surface is in direct contact (2). Peritubular cells, together with collagen fibrils and other extracellular matrix (ECM)¹ components in

the boundary tissue, constitute the tubular wall. This complex structure provides a nonspecific barrier that prevents penetration of blood vessels and limits the passage of cells and macromolecules into the tubule (3).

When maintained in culture, Sertoli cell-enriched aggregates plated on top of a multilayer of peritubular cells initially spread to form a monolayer. Subsequently, a restructuring occurs in which Sertoli cells re-aggregate to form mounds, and then nodules (4). The nodules become surrounded by ribbons of peritubular cells, separated by a limiting membrane whose structure is similar to that of the lamina propria. The

¹ *Abbreviations used in this paper:* ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; FIRT, follicle-stimulating hormone, insulin, testosterone, and retinol; MEM, Eagle's modified minimum essential medium.

co-cultured cells eventually form complex protrusions, caused by the merging of adjacent nodules. The morphology of these protrusions which develop *in vitro* grossly resembles the histologic appearance of the seminiferous tubule, and the processes involved appear to be specific to cell types described (4). In contrast, Sertoli cells or peritubular cells in monoculture spread to form relatively uniform layers.

We postulate that interactions between Sertoli cells and peritubular cells in co-culture are related to those occurring *in vivo*, in which case the co-cultured system could provide a useful model for the investigation of the formation and deposition of seminiferous tubular ECM components, including the basal lamina. In approaching this problem, we initially have determined the levels of various ECM proteins produced by populations of isolated Sertoli cells or peritubular cells, maintained in monoculture and in co-culture under different experimental conditions. Results we present in this article demonstrate that peritubular cells and Sertoli cells in monoculture produce different ECM components, both qualitatively and quantitatively, and that most of the ECM components appear to remain in the soluble form in monocultures maintained in serum-free medium. Other experiments described show that the two cell types in co-culture act cooperatively to facilitate the deposition of extracellular fibrils.

MATERIALS AND METHODS

Cell Preparation and Culture Procedures: Purified Sertoli cell preparations were isolated from testes of 20-d-old Wistar rats (Canadian Breeding Laboratories, Montreal) using trypsin, collagenase, and hyaluronidase in combination with mechanical agitation (5). For sparse cultures, purified Sertoli cell preparations were seeded on glass coverslips or polystyrene culture dishes at 400 aggregates/100 mm², with each aggregate containing 20–40 cells. For confluent cultures they were plated at 1,500 aggregates/100 mm². Unless otherwise specified the medium and culture conditions employed were identical to those previously described (6). Peritubular cells were prepared from testes of 20-d-old Wistar rats, according to procedures described by Tung and Fritz (7), and were cultured in the presence of 10% calf serum in Eagle's modified minimum essential medium (MEM) for 5 d. We then subcultured peritubular cells by removing the cells with trypsinization and plating at ~5,000 cells/100 mm² in 10% calf serum in MEM. Subcultured peritubular cells were grown for 4 d in MEM containing 10% calf serum, at which time the medium was removed and the cells were washed for 6 h in serum-free MEM. At this time, serum-free MEM was replenished, and this was designated as time zero of serum free peritubular cell culture. For co-cultures, Sertoli cell aggregates were seeded on top of the subcultured peritubular cells, in serum-free MEM at a density of 400 aggregates/100 mm², corresponding approximately to an equal ratio of Sertoli cells to peritubular cells (4). The purity (>95%) of cell preparations and the morphological characteristic of cells in culture were the same as those described previously (4–7).

Antibodies: We purchased antisera against rat fibronectin from Calbiochem-Behring Corp. (La Jolla, CA). Mouse monoclonal antibody (IgG) directed against porcine plasma fibronectin was a gift from Dr. J. Aubin, University of Toronto. Polyclonal antisera (generously provided by Dr. J. Sodek, University of Toronto) were raised in sheep against Type I and against Type III collagens isolated from porcine gingival tissue (8). Sheep antiserum against mouse Type IV collagen was a gift from Dr. H. Kleinman of the National Institute of Dental Research (Bethesda, MD). These antisera were purified by affinity adsorption on minicolumns (9) containing relevant antigens coupled to CNBr-Sepharose 4B (Pharmacia, Uppsala, Sweden). After the affinity resin (200 μ l) was washed with 1% wt/vol bovine serum albumin in phosphate-buffered saline [PBS] containing 0.05% vol/vol Tween 20 (PBS-Tween), 80 μ l of the antiserum was applied. The resin was washed five times with 100- μ l aliquots of PBS-Tween before the bound antibodies were eluted with 0.05 M glycine-HCl buffer, pH 2.5. The eluted antibodies were neutralized immediately with 1 M Tris-HCl, pH 9.0, and then dialyzed against PBS at 4°C. Polyclonal rabbit antiserum directed against mouse laminin was generously provided by Dr. H. Kleinman.

Bulk adsorption was carried out following procedures previously described (5). Matrix components used for adsorption included rat plasma fibronectin (Calbiochem-Behring Corp.), porcine Type I and III collagens (provided by Dr.

J. Sodek), and Type IV collagen (provided by Dr. Sodek).

Immunofluorescent Techniques: Sparse monocultures or co-cultures grown on glass coverslips were fixed after various periods of culture with periodate-lysine-paraformaldehyde (10), then treated with acetone at -10°C for 7 min. Preparations were then washed with multiple changes of PBS at 4°C. The specimens were incubated with the antibody solution in serial dilutions in PBS (and at concentrations indicated in the legend to Fig. 1) and were again washed with PBS containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO). Affinity purified F(ab)₂ fragments of rabbit or goat immunoglobulins conjugated with fluorescein isothiocyanate (Cappel Laboratories, West Chester, PA) diluted 1:20 with PBS were added to the washed specimens for another 30 min and followed by additional washes with PBS containing 0.05% Tween 20. The preparation was mounted and examined with fluorescent microscopy as previously described (11).

Enzyme-linked Immunosorbent Assays: Nonequilibrium, competition enzyme-linked immunosorbent assays (ELISA) were performed by slightly modified procedures described by Rennard et al. (12). Wells of Immulon microelisa plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 100 μ l/well of the antigen (2 mg/ml) in 20 mM sodium carbonate, pH 9.6, overnight at 4°C. After plates were coated they were washed three times in PBS-Tween 20 (150 mM NaCl, 20 mM Na₂HPO₄, pH 7.4, containing 0.05% Tween 20) before 100 μ l of the preincubated antigen-antibody solutions was applied. Various dilutions of the antigen (standard curve or sample) were incubated overnight in an equal volume of a fixed amount of antibody, that was optimized for each ECM component. Antigen dilutions were made using MEM. All antibody dilutions were prepared in PBS-Tween. Plate-to-plate variation in the assays necessitated inclusion of a standard curve for each set of determinations. After incubation for 30 min at room temperature with the antigen-antibody solutions, plates were washed three times in PBS-Tween and followed by addition of the alkaline phosphatase conjugated second antibody. Plates were then washed three times in PBS-Tween and once in diethanolamine buffer (5 mM MgCl₂, 10% diethanolamine, pH 9.8). A fresh solution of 1 mg/ml *p*-nitrophenyl phosphate (phosphatase substrate; Sigma Chemical Co.) in diethanolamine buffer was then added to the wells. Color development occurred within 1 h, and plates were monitored at 405 nm with a Titertek Multiscan densitometer (Organon Teknika, Helsinki). Specific conditions for each ECM component are described below. Human laminin and rabbit anti-mouse laminin were received from Dr. H. Kleinman. Anti-laminin was used at a final dilution of 1:10,000 and anti-rabbit IgG conjugate was used at 1:500 for 1 h. Human fibronectin was purchased from Sigma Chemical Co. Goat anti-rat fibronectin (cross-reactive with human fibronectin Calbiochem-Behring Corp.) was used at a final dilution of 1:20,000. Incubation with anti-goat IgG conjugate (1:500; Sigma Chemical Co.) was for 1 h. Rat tail collagen I was received from Sigma Chemical Co. Collagen I antiserum raised in sheep was used at 1:500 dilution. Anti-goat conjugate was used at 1:1,000 for a 45-min incubation. Collagen IV and sheep anti-collagen IV (used at 1:200) were a gift from Dr. H. Kleinman. Anti-goat conjugate diluted 1:1,000 was used for a 35-min incubation.

RESULTS

Evidence that Fibronectin Is Produced by Peritubular Cells but Not by Sertoli Cells in Culture

IMMUNOCYTOCHEMICAL OBSERVATIONS ON LOCALIZATION OF FIBRONECTIN: In confirmation of previous observations (5), peritubular cells in monoculture exhibit a strongly positive immunofluorescent reaction to fibronectin antibody. Most of the fibronectin is distributed in the perinuclear region of peritubular cells maintained in serum-free medium for 2 to 6 d (Fig. 1A). We obtained identical results with goat anti-rat fibronectin and with mouse monoclonal anti-pig fibronectin. Fibronectin antibody-reactive material is not detectable in primary cultures of purified Sertoli cell-enriched preparations maintained under comparable conditions (Fig. 1B). In co-cultures of the two cell types, immunofluorescence dependent upon fibronectin is more intense than that visualized in monocultures of peritubular cells. The fibronectin is extensively deposited extracellularly in a fibrillar arrangement (Fig. 1C). Fibronectin antibody preadsorbed with fibronectin did not react with either cell type, whereas

antibody preadsorbed with laminin or collagen showed the same distribution as nonadsorbed fibronectin antibody (data not shown).

IMMUNOCHEMICAL DETERMINATION OF LEVELS OF FIBRONECTIN IN THE CULTURE MEDIUM: The competitive ELISA assay employed was linear between 30 and

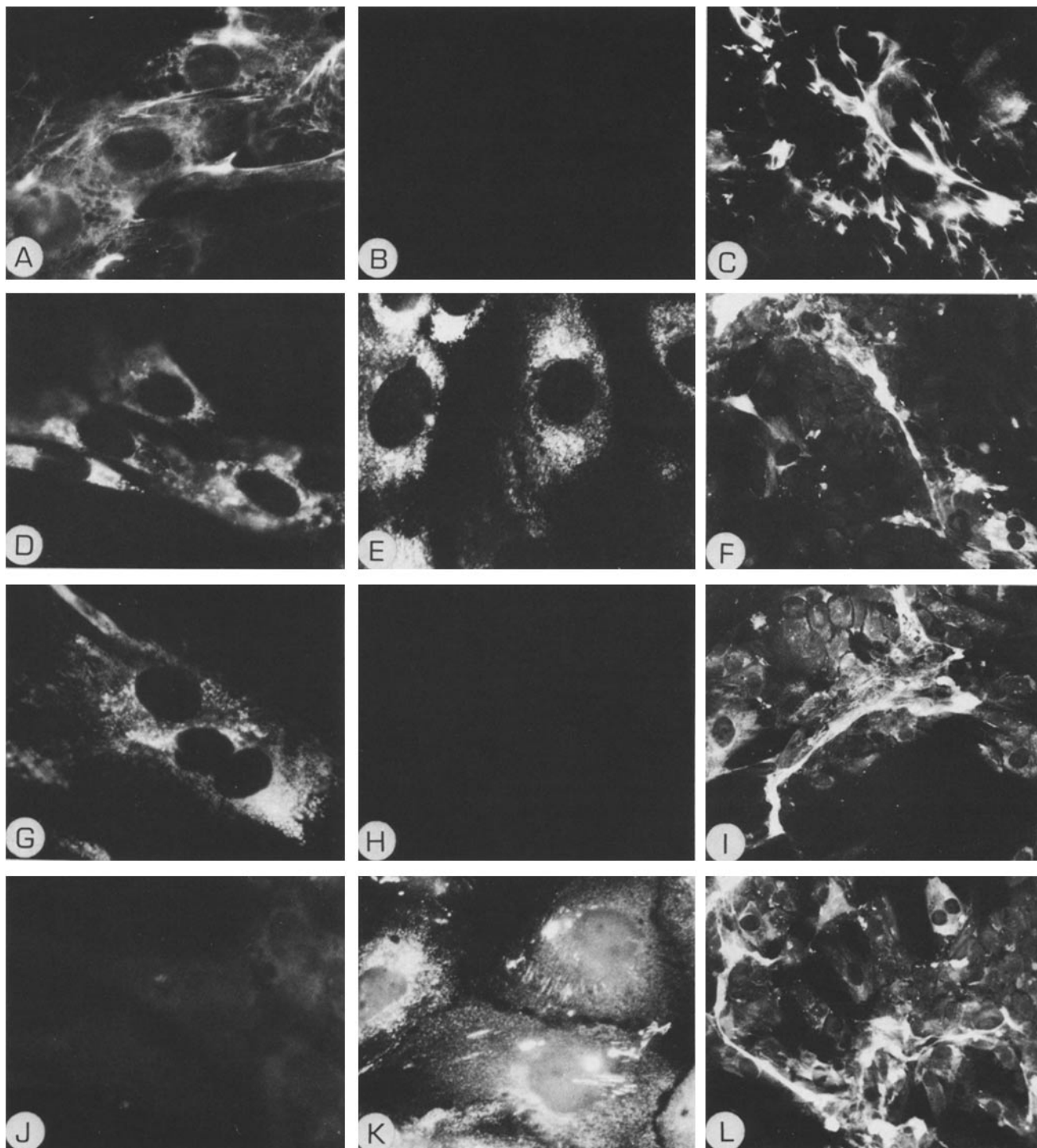


FIGURE 1 Specific immunolocalization of ECM components in secondary sparse monocultures on glass coverslips of peritubular cells (A, D, G, and J); primary sparse monoculture of Sertoli cells (B, E, H, and K); and Sertoli cell-peritubular cell co-cultures (C, F, I, and L). Cells were maintained in serum-free MEM containing 50 μ g/ml ascorbic acid for 4 d, then fixed and processed for indirect immunofluorescent microscopy by the use of undiluted mouse monoclonal IgG against porcine plasma fibronectin from hybridoma culture medium (A-C); affinity purified sheep anti-porcine Type I collagen at a dilution of 1:10 (G-I); affinity purified sheep anti-mouse Type IV collagen at a dilution of 1:10 (D-F); or polyclonal rabbit antiserum against mouse laminin at a dilution of 1:15 (J-L). Note that immunofluorescent intensities of extracellular ECM components observed in co-cultures are much greater than intensities of perinuclear ECM components (C, F, I, and L). In monocultures, extracellular ECM components are not detectable, whereas the immunofluorescent intensity of perinuclear ECM components becomes readily evident at higher magnifications shown (A, D, E, G, and K). (A, B, D, E, G, H, J, and K) \times 400; (C, F, I, and L) \times 200.

500 ng fibronectin/well under conditions used, and the antibody did not react with laminin, Type I collagen, or Type IV collagen (data not shown). Levels of soluble fibronectin in the medium in which peritubular cells had been maintained in monoculture tended to be somewhat higher during the first two days (560 ng/ μ g cell DNA) than during days four to six (432 ng/ μ g cell DNA) (Table I). No fibronectin was detectable in the medium in which Sertoli cells had been maintained in monoculture (Table I). When expressed as nanograms fibronectin per microgram total cell DNA, the amounts of fibronectin present in the medium in which both cell types had been maintained in co-culture were about one-half of the values determined in peritubular cell monocultures (Table I). Approximately equivalent numbers of Sertoli cells and peritubular cells were present in the co-cultured system. If it is assumed that Sertoli cells continued to produce no fibronectin and that levels of fibronectin detected represented only that synthesized by the peritubular cells in co-culture, the recalculated values (expressed as nanograms per microgram peritubular cell DNA) are not substantially different from levels of fibronectin observed in peritubular cells during the first 96 h of monoculture (Table I).

Addition of a mixture of hormones (follicle-stimulating hormone, insulin, and testosterone) and retinol (FIRT) to the culture medium did not increase levels of soluble fibronectin produced by peritubular or Sertoli cell preparations in monoculture or co-culture (Table I). In the FIRT-treated co-cultured system, soluble fibronectin levels were lower during the 96- and 144-h periods of culture than in the nontreated preparations (Table I). This difference was also observed in the 144-h collection in FIRT-treated peritubular cell monocultures (Table I).

Evidence that Type I Collagen Is Produced by Peritubular but Not Sertoli Cells during the First 6 d of Culture

IMMUNOCYTOCHEMICAL OBSERVATIONS ON LOCALIZATION OF TYPE I COLLAGEN: To determine the localization of collagen in peritubular cells and Sertoli cells maintained in monoculture or co-culture for 6 d we performed experiments comparable to those reported above for fibronectin. Peritubular cells in monoculture gave a positive immunofluorescent reaction to Type I collagen antibody (Fig. 1 G). The immunofluorescence was more intense in the co-cultured system, with obvious extracellular deposition (Fig. 1 I). Sertoli cells in monoculture or co-culture had no detectable reaction to the Type I collagen antibody (Fig. 1, H and J). This antibody reaction became negative against peritubular cells when the antibody was preadsorbed with Type I collagen, but not when it was preadsorbed with Type IV collagen, laminin, or fibronectin (data not shown).

IMMUNOCHEMICAL DETERMINATION OF SOLUBLE TYPE I COLLAGEN IN THE CULTURE MEDIUM: The competitive ELISA assay employed was linear between 30 and 500 ng Type I collagen per well under conditions described, and was not reactive with laminin, fibronectin, or Type IV collagen (data not shown). Levels of soluble Type I collagen present in the medium in which peritubular cells had been maintained in monoculture in the presence or absence of FIRT ranged between 210 to 276 ng/ μ g cell DNA during three successive 48-h collection periods (Table II). No Type I collagen was detectable in medium in which Sertoli cells had

TABLE I
Levels of Soluble Fibronectin Produced by Peritubular Cells or Sertoli Cells Maintained in Monoculture or Co-culture

Cell type in culture	Additions to medium	Levels of fibronectin in medium during successive 48-h collections		
		0-48 h	48-96 h	96-144 h
		ng/ μ g DNA		
Peritubular cells	Control	560 \pm 27	475 \pm 21	432 \pm 23*
	FIRT	517 \pm 25	425 \pm 19	353 \pm 18*
Sertoli cells	Control	0	0	0
	FIRT	0	0	0
Peritubular and Sertoli cells	Control	240 \pm 11	244 \pm 13 [‡]	182 \pm 11 [‡]
	FIRT	248 \pm 13	176 \pm 8.7 [‡]	121 \pm 8.7 [‡]
Peritubular and Sertoli cells	Control	480 [§]	488 [§]	364 [§]
	FIRT	496 [§]	352 [§]	248 [§]

FIRT represents the addition of follicle-stimulating hormone (NIH, S16, 100 ng/ml), insulin (5 μ g/ml), retinol (0.35 μ M), and testosterone (1 μ M).

* Statistically different values (* P < 0.01 and [‡] P < 0.05) between control cells and FIRT-treated cells at times shown.

[§] Data are calculated as nanograms fibronectin per microgram peritubular cell DNA on the assumption that no fibronectin was produced by Sertoli cells. All data are expressed as the mean \pm SEM for three separate experiments, each of which was analyzed in triplicate (n = 9).

TABLE II
Levels of Soluble Type I Collagen Produced by Peritubular Cells or Sertoli Cells Maintained in Monoculture or Co-culture

Cell type in culture	Additions to medium	Levels of collagen I during successive 48-h collections		
		0-48 h	48-96 h	96-144 h
		ng/ μ g DNA		
Peritubular cells	Control	276 \pm 14*	223 \pm 9.3	254 \pm 12*
	FIRT	210 \pm 13*	226 \pm 31	211 \pm 8.0*
Sertoli cells	Control	0	0	0
	FIRT	0	0	0
Peritubular and Sertoli cells	Control	312 \pm 17	138 \pm 11*	96 \pm 5.0*
	FIRT	277 \pm 13	74 \pm 9.0*	78 \pm 4.3*
Peritubular and Sertoli cells	Control	624 [‡]	276 [‡]	192 [‡]
	FIRT	554 [‡]	148 [‡]	156 [‡]

FIRT concentrations are as described in Table I.

* Statistically different values (P < 0.01) between control cells and FIRT-treated cells at times shown.

[‡] Nanograms per microgram peritubular cell DNA on the assumption that no Type I collagen was produced by Sertoli cells. All data are expressed as the mean \pm SEM for three separate experiments, each of which was analyzed in triplicate (n = 9).

been maintained in monoculture under all conditions examined during the first 6 d of culture (Table II).

During the initial 48 h of co-culture, levels of Type I collagen produced (312 ng/ μ g total cell DNA, or an estimated 624 ng/ μ g peritubular cell DNA) were considerably higher than the amounts of collagen released into the medium by the two cells maintained in monoculture (276 ng/ μ g peritubular cell DNA) (Table II). These findings suggest that the production of Type I collagen by peritubular cells was probably stimulated by the presence of Sertoli cells in the co-cultured system. Since no Type I collagen was detected immunocytochemically in Sertoli cells maintained in monoculture or co-culture for 6 d (Fig. 1, H and J), it appears unlikely that the presence of peritubular cells stimulated Sertoli cells to produce this protein. However, Type I collagen was pro-

duced by Sertoli cells maintained for more than 10 d in monoculture (80 ± 20 ng/ μ g Sertoli cell DNA during the period from 10 to 12 d). Although long-term monocultures of Sertoli cell-enriched preparations produced Type I collagen, they did not form detectable amounts of fibronectin (data not shown). The decreased levels of Type I collagen in the medium of the co-cultured system during the second and third collection periods (Table II) were associated with an increased deposition of this protein in extracellular fibrils (Fig. 1J). Addition of the hormone mixture elicited a small but statistically significant decrease in levels of soluble Type I collagen in the medium during the 96- and 144-h collection periods in the co-cultured system, and during the 48- and 144-h collection periods in monocultures of peritubular cells (Table II).

Evidence that Type IV Collagen Is Produced by Peritubular and Sertoli Cells in Culture

IMMUNOCYTOCHEMICAL OBSERVATIONS ON THE LOCALIZATION OF TYPE IV COLLAGEN: Type IV collagen detectable by specific reactivity with the antibody to this protein is evident in peritubular cells and in Sertoli cells maintained in monoculture or co-culture (Fig. 1, D-F). The strongest immunofluorescence, especially in extracellular fibrils, is evident in the co-cultured system (Fig. 1F). The antibody against Type IV collagen preadsorbed with Type IV collagen was nonreactive with either cell type, whereas the antibody preadsorbed with Type I collagen, fibronectin, or laminin reacted in a manner indistinguishable from that observed with the unadsorbed antibody (data not shown).

IMMUNOCHEMICAL DETERMINATION OF LEVELS OF SOLUBLE TYPE IV COLLAGEN IN THE CULTURE MEDIUM: The competitive ELISA assay employed was linear between 15 and 500 ng Type IV collagen/well under conditions described and was not reactive with Type I collagen, fibronectin, or laminin (data not shown). Levels of soluble Type IV collagen in the medium in which Sertoli cells or peritubular cells had been maintained in monoculture, in the presence or absence of FIRT, varied between 300 and 436 ng/ μ g DNA during each of three successive 48-h collection periods (Table III). In the co-cultured system, levels of Type IV collagen in the medium were lower, ranging between 169 and 256 ng/ μ g total DNA per 48 h in cultures maintained in

the presence or absence of FIRT (Table III). Since more Type IV collagen appeared in extracellular fibrils in the co-cultured system (Fig. 1F) than in either of the monocultures (Fig. 1, D and E), the diminished levels of soluble Type IV collagen observed during co-culture (Table III) appear to be associated with an increased deposition of Type IV collagen under these conditions. FIRT-treated cells tended to have lower levels of soluble Type IV collagen during several but not all collection periods (Table III).

Addition of aminopropionitrile (1.4 mM), an agent that prevents cross-linking of collagen *in vivo* (13), and *in vitro* (14), did not alter levels of soluble collagen measured under conditions otherwise identical to those reported in Tables I-III for cultures of peritubular cells and Sertoli cells (data not shown).

Production of Laminin by Peritubular Cells and by Sertoli Cells in Culture

IMMUNOCYTOCHEMICAL OBSERVATIONS ON THE LOCALIZATION OF LAMININ: Immunofluorescence dependent on reactivity with a specific antibody against laminin is detectable in Sertoli cells maintained in serum-free culture (Fig. 1K), but not in peritubular cells (Fig. 1J). Extracellular deposition is scant or nondetectable in monocultures (Fig. 1, J and K), but laminin antibody-reactive material is clearly evident in extracellular fibrils in co-cultures of Sertoli and peritubular cells (Fig. 1L). The antibody preadsorbed with laminin was nonreactive with either cell type, whereas preadsorption with fibronectin, Type I collagen, or Type IV collagen did not alter its reactivity (data not shown).

IMMUNOCHEMICAL DETERMINATION OF LEVELS OF SOLUBLE LAMININ IN THE CULTURE MEDIUM: The competitive ELISA assay employed was linear between 30 and 250 ng laminin/well under conditions employed and was nonreactive with comparable levels of fibronectin, Type I collagen, or Type IV collagen (data not shown). In non-concentrated samples of medium in which Sertoli cells or peritubular cells had been maintained in monoculture or co-culture, laminin levels were below detectable limits.

DISCUSSION

Data presented in this communication demonstrate that peritubular cells and Sertoli cells synthesize components required for ECM and basal lamina formation. Sertoli cells do not release fibronectin or Type I collagen into the medium when maintained in serum-free culture for periods of up to 6 d, but they produce appreciable levels of Type IV collagen and relatively small amounts of laminin. Peritubular cells in culture release fibronectin, Type I collagen, and Type IV collagen into the medium. Deposition of the ECM proteins as extracellular fibrils is minimal in monocultures maintained in the absence of serum, but deposition becomes appreciable in the co-cultured system. Addition of serum results in the increased deposition of ECM components, such as fibronectin, in extracellular fibrils by monocultures of peritubular cells (5). We have noted comparable effects of serum on the deposition of collagen (unpublished observations). It is possible that the two cell types in co-culture contribute factors similar to those in serum which enhance ECM deposition.

It appears likely that epithelial (Sertoli) cell-mesenchymal (peritubular) cell interactions observed in the testis may be

TABLE III

Levels of Soluble Type IV Collagen Produced by Peritubular Cells or Sertoli Cells Maintained in Monoculture or Co-culture

Cell type in culture	Additions to medium	Levels of collagen IV during successive 48-h collections		
		0-48 h	48-96 h	96-120 h
		ng/ μ g DNA		
Peritubular cells	Control	436 \pm 25*	390 \pm 20	350 \pm 18*
	FIRT	354 \pm 20*	370 \pm 16	300 \pm 12*
Sertoli cells	Control	419 \pm 24*	332 \pm 17	322 \pm 17
	FIRT	326 \pm 21*	328 \pm 15	336 \pm 18
Peritubular and Sertoli	Control	208 \pm 11*	217 \pm 10*	197 \pm 10*
	FIRT	256 \pm 13*	170 \pm 7.3*	169 \pm 7.7*

FIRT concentrations are as described in Table I. All data are expressed as the mean \pm SEM for three separate experiments, each of which was analyzed in triplicate ($n = 9$).

* Statistically different values (* $P < 0.05$ and * $P < 0.01$) between control cell and FIRT-treated cells at times shown.

homologous to epithelial cell-mesenchymal cell interactions previously described in other systems in which basal lamina formation has been investigated. For example, renal epithelial and mesangial cells each synthesize Type IV collagen (15), just as Sertoli cells and peritubular cells do (Table III). Both glomerular epithelial and mesangial cells produce proteoglycans, but epithelial cells synthesize predominantly heparan sulfate proteoglycans and mesangial cells produce predominantly chondroitin sulfate proteoglycans (15, 16). Sertoli cell-enriched preparations synthesize proteoglycans that contain both chondroitin sulfate and heparan sulfate (17; Skinner, M. K., and I. B. Fritz, manuscript submitted for publication). In contrast, peritubular cells synthesize only proteoglycans rich in chondroitin sulfate (Skinner, M. K., and I. B. Fritz, manuscript submitted for publication). Both epithelial and mesangial cells of the glomerulus produce fibronectin (18, 19). In the testis, however, peritubular cells synthesize fibronectin *in vitro*, but Sertoli cells do not (Table I and Fig. 1) (5).

In co-cultures of Sertoli cells and peritubular cells, lower levels of soluble Type I collagen and fibronectin were detectable in medium from the 48-h collection on day 6 than in medium from the initial 48-h collection (Tables I and II). In contrast, levels of soluble Type I collagen secreted by monocultures of peritubular cell were relatively constant during the three 48-h collections (Table II). Levels of soluble ECM components analyzed were lower in the medium from co-cultures than in the medium from monocultures during the third 48-h collection on day 6 (Tables I-III). These data suggest an increased deposition of ECM components in extracellular fibrils in the co-cultured system. Further evidence that the two populations of cells may act synergistically to elicit the deposition of soluble ECM components secreted by each of the two cell types is provided by the immunocytochemical localization of ECM components in extracellular fibrils (Fig. 1). The interpretation offered is compatible with previous observations that basal lamina formation takes place when peritubular cells and Sertoli cells are in co-culture, but a basal lamina is not evident in monocultures of either cell type (4).

The only change noted in cells maintained in the presence of a mixture of FIRT was a tendency towards decreased levels of soluble ECM components, particularly in the medium of cells maintained in co-culture from 48 to 144 h (Tables I-III). Effects noted are small, but they suggest that the addition of FIRT may have elicited increased deposition of these ECM components as fibrils. Additional experiments are required to test this supposition, since it is possible that FIRT may have acted to decrease the total synthesis of fibronectin or Type I collagen.

The cooperativity demonstrated in this communication between Sertoli cells and peritubular cells in the production and deposition of ECM components in co-culture may be related to other interactions observed to occur between these two cell types. For example, Sertoli cells permit peritubular cells to survive for months in a chemically defined medium, whereas peritubular cells in monoculture require the presence of serum for survival (4). In turn, peritubular cells permit Sertoli cells to survive longer and to maintain function as measured by the sustained production of androgen-binding protein (4, 20). Peritubular cells in monoculture also secrete protein(s) (P Mod-S, with an apparent *M_r* of 70,000) into the medium which stimulate the synthesis of androgen-binding protein, transferrin, and certain other products by primary

cultures of Sertoli cells (21). Androgens, which are required *in vivo* for the normal development of peritubular cells (22) and for the maintenance of spermatogenesis (23), have been observed to stimulate primary cultures of peritubular cells to secrete higher levels of P Mod-S into the medium (21).

These interactions between Sertoli cells and peritubular cells in co-culture appear to have some degree of specificity, since the restructuring reported during co-culture cannot be duplicated by substituting other cell types examined (4), and the Sertoli cell stimulatory factor present in peritubular cell conditioned medium is not produced by other cells investigated, such as 3T3 cells (21). Sustained histotypic expression of Sertoli cell characteristics *in vitro* is favored when Sertoli cells are cultured on an extracellular matrix derived from the seminiferous tubule (24). This is not the case when Sertoli cells are cultured on an extracellular matrix derived from liver (Tung, P. S., and I. B. Fritz, unpublished observations). Possible functions of the ECM synthesized by Sertoli cells and peritubular cells in the seminiferous tubule include those which are common to ECM in other tissues, such as influences on the shape, mobility, and expression of the differentiated phenotype of cells in contact with matrix components (25). Specialized functions may exist that are associated with the unique organization of the seminiferous tubule, and with changes during the restructuring that occurs during spermatogenesis (26, 27). We have discussed elsewhere the possible role of proteases, such as plasminogen activator secreted by Sertoli cells at discrete stages of the cycle of the seminiferous epithelium, in these processes (28, 29). We are exploring the hypothesis that Sertoli cells and adjacent peritubular cells may function cooperatively during tubulogenesis and during various stages of spermatogenesis to modify the structure of the basal lamina, and the nature of ECM components deposited. The possible relevance of observations presented to the restructuring of the seminiferous tubule which occurs as clones of spermatocytes are translocated to the adluminal compartment (27) remains to be determined.

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