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Acute Effect of Vasectomy on the Function of the Rat Epididymal Epithelium and Vas Deferens

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Abstract

Persistent infertility after apparently successful vasectomy reversal is common. One possible etiology is epididymal epithelial dysfunction resulting in improper sperm maturation after vasectomy reversal. The epididymal epithelium secretes a number of proteins that are thought to be required for the maturation of sperm. Ligation of the vas deferens during vasectomy may affect the synthesis of some of these proteins. In the present study, the function of the epididymal epithelium was assessed at early times after vasectomy (1, 4, and 7 days) by measuring the level of mRNA of 4 secreted proteins: Crisp-1, clusterin, osteopontin, and transferrin. In addition, the site of synthesis of these proteins was determined by immunocytochemistry. The results demonstrated that the expression of Crisp-1 and clusterin, representative epididymal secretory proteins, was largely unaffected by vasectomy. However, osteopontin mRNA increased in the vas deferens in response to vasectomy. Immunocytochemical localization of osteopontin suggested that both infiltrating immune cells and deferential luminal epithelium were responsible for this up-regulation. Transferrin expression was viewed as a marker for immune cells at the site of injury. However, both the caput epididymis and deferential epithelia were found to express transferrin, in addition to immune cells. In conclusion, there appear to be only minor changes in expression of genes encoding

epididymal secretory proteins acutely after vasectomy, but, not surprisingly, there was evidence of an inflammatory response after vasectomy.

Key words: Crisp-1, clusterin, osteopontin, transferrin

Vasectomy is a commonly performed procedure for birth control. Historically, the consequences of vasectomy have been permanent. However, with advancement of surgical technique, methods have been developed to reverse this previously permanent procedure. By the late 1970s both macroscopic and microscopic surgical techniques were being utilized for vasectomy reversal, with success rates similar to those of today (Silber, 1977; Kabalin and Kessler, 1991). In approximately 90% of cases vasovasostomy restores the patency of the vas deferens as measured by return of sperm to the ejaculate. However, only about 50% of cases are clinically successful as determined by achievement of pregnancy (Belker et al, 1991; Feber and Ruiz, 1999).

Many factors may contribute to persistent infertility after successful reanastomosis of the vas deferens. These factors include immunologic responses, such as local inflammation and granuloma formation (Flickinger et al, 1995), and the presence of high titers of serum antisperm antibodies (Kay et al, 1993). The length of time the vas deferens has been disrupted may also contribute to the ultimate failure of an apparently successful reversal (Yamamoto et al, 1997; Abdelmassih et al, 2002). It is also possible that changes in the function of the epididymal epithelium may occur with time after vasectomy, resulting in poor sperm quality after vasovasostomy, as suggested by studies in both humans and animals (Flickinger et al, 1995; Doiron et al, 2003; Legare et al, 2004).

The epididymis has 2 primary functions. First, the cauda epididymis serves as the primary storage site for sperm prior to ejaculation. Second, the epididymal epithelium secretes proteins and other components that create the specific luminal microenvironment required for the maturation of sperm (Hinton and Palladino, 1995; Kirchhoff et al, 1997). Some of the glycoproteins secreted by the epididymis participate in the maturation process by being incorporated into the plasma membrane of maturing spermatozoa (Kirchhoff et al, 1997). Many of these proteins are secreted under the influence of androgens and testicular fluid components (Brooks and Higgins, 1980; Turner et al, 1995). Ligation of the vas deferens during vasectomy may affect the microenvironment of the epididymis through mechanical pressure on the epithelial cells, affecting their function directly, or by causing cessation of flow in the tubule, thereby decreasing delivery of required testicular factors and androgens (Johnson and Howards, 1975).

In the study reported herein, the function of the epididymal epithelium was assessed after vasectomy by determining the level of mRNA of 4 secreted proteins and immunocytochemically staining the tissue to localize the site of synthesis of the translation products. Relatively early time points were chosen to determine if the acute effects of ligation, such as increased luminal pressure, caused epithelial dysfunction prior to "blowout" of the epididymal tubule. The results demonstrate that the expression of representative epididymal secretory protein mRNAs was largely unaffected by vasectomy. However, there was a substantial accumulation of osteopontin mRNA and protein in the vas deferens in response to vasectomy. Immunocytochemical localization of osteopontin suggests that both infiltrating immune cells and the deferential luminal epithelium were responsible for this up-regulation. Immunocytochemical localization of transferrin suggests an inflammatory response to vasectomy, with transferrin secretion within the granuloma and up-regulation of deferential luminal epithelial secretion close to the granuloma. The implications of this response to vasectomy are discussed.

Materials and Methods

Experimental Rat Vasectomy

Four-month-old male Sprague Dawley rats were obtained from Harlan Sprague-Dawley (Indianapolis, Ind) and housed with a 12-hour light:dark cycle. Rat chow and water were provided ad libitum. After induction of anesthesia (intraperitoneal injection of ketamine [45 mg/kg] and xylazine [5 mg/kg]), the abdomen was shaved and swabbed with Betadine. A 1-cm midline incision was made in the abdomen, and bilateral vasa deferentia were retrieved and exposed. Using 4.0 silk suture, the vas deferens was serially ligated and subsequently transected between the ligatures. Each end was then replaced within the abdominal cavity, and the procedure was repeated on the other side. The incision was closed in 2 layers with 4.0 silk suture, in a running fashion. The procedure was duplicated for the sham animals. Briefly, the incision was made, vasa deferentia were exposed, 4.0 silk was passed, but the vasa deferentia were not ligated or transected. The incision was closed in the same manner. Normal control animals received no surgical manipulation.

Normal, sham, and experimental animals were anesthetized by an intraperitoneal injection of pentobarbital and then decapitated at 1, 4, and 7 days after vasectomy. The abdominal aorta was isolated and cannulated. The animals were perfused with 50 mL of PBS to clear blood from the vasculature. One side of the reproductive tract was removed and testis, epididymis, and vas deferens separated. The epididymis was further divided into caput, corpus, and cauda regions. All tissues were snap-frozen in liquid nitrogen and stored at −80 °C. The rat was then perfusion fixed with 50 mL of Bouin solution and the contralateral reproductive tract was removed, embedded in paraffin, and sectioned for histology and immunocytochemistry. All animal experimental procedures were reviewed and approved by the Animal Care and Use Committee at the University of Minnesota.

Northern Blot Analysis

To obtain a measurement of the average level for each mRNA measured in this study, equal percent aliquots of tissue homogenates were combined and total RNA was isolated from the mixture using the method of Chomcynski and Sacchi (Chomczynski and Sacchi, 1987). Four animals were used in the normal (N) group, in the sham group, and in the groups at 1 and 4 days postvasectomy. Due to granuloma formation by 7 days postvasectomy, 2 additional animals were included at this time point. Subsequently, 4 of 6 animals contained granulomas. Homogenates from all 6 animals from day 7 postvasectomy were combined to make the testis, caput, corpus, and cauda samples. The vas deferens tissue of the 6 animals at day 7 was separated into 4 different samples. Homogenates from the 2 animals without granuloma were combined. Two of the animals with granulomas had the tissue separated into vas deferens only and granuloma only. The final 2 animals with granulomas had the vas deferens tissue and granuloma tissue combined. The RNA was quantified by spectrophotometry, and 10 µg aliquots of total RNA were separated by electrophoresis on 1% agarose-formaldehyde gels, transferred to nylon membranes by capillary blotting (Sambrook et al, 1989), and cross-linked to the membrane by UV irradiation using a Stratalinker (Stratagene, La Jolla, Calif). The membranes were prehybridized for 1 hour in hybridization buffer (5% SDS, 400 mM $NaPO_4$, 1 mM EDTA, 1 mg/mL BSA, 50% formamide), followed by hybridization with [^{32}P] dCTP-labelled cDNA probes synthesized using the Rad Prime Kit (Life Technologies, Rockville, Md) according to the manufacturer's instructions. Hybridizations were carried out under high-stringency conditions at 45 °C overnight. After washing (2 x SSC, 0.1% SDS at room temperature and repeated at 42 °C, then 0.1 x SSC, 0.1% SDS, 5 mM EDTA at 42 °C and repeated at 50 °C) the blots were visualized by exposure to X-Omat film (Kodak Co, Boston, Mass) at −80 °C. Membranes were stripped and sequentially probed with all 5 cDNAs used in this study. Northern blot

analyses were repeated at least twice for each cDNA probe. The cDNA clones for rat osteopontin and Crisp-1 were gifts from Dr David Hamilton, University of Minnesota, and the cDNA clones for rat transferrin and clusterin (SGP-2) were gifts from Dr Michael Griswold, Washington State University. The cDNA probe for GAPDH was purchased from Ambion (Austin, Tex).

Immunocytochemistry

Paraffin embedded tissue was immunologically stained with MPIIIB101, a monoclonal anti-rat osteopontin antibody from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, Iowa) and rabbit anti-rat transferrin antibody purchased from Research Diagnostics (Flanders, NJ). Briefly, tissue sections were deparaffinized with xylene and rehydrated to PBS through a series of decreasing ethanol washes. To optimize antigen presentation, the tissue sections were then incubated in 10 mM citric acid for 30 minutes at 100 °C. The tissue was blocked with 1% BSA in PBS for 30 minutes, followed by incubation in primary antibody (1:100 dilution for transferrin and 1:50 dilution for osteopontin) in PBS for 1 hour at room temperature. Tissue sections were then washed and incubated with the appropriate FITC-labeled second antibody (Sigma, St. Louis, Mo) at 1:100 dilution for 1 hour at room temperature. Control sections were incubated with secondary antibody only. Slides were coverslipped and viewed with a Nikon Eclipse TE300 inverted microscope. Digital images were captured with an attached Hamamatsu Digital CCD Camera and processed using the C-Imaging Systems Simple PCI software (Cranberry Township, Pa). In order to adequately appreciate structures on the control images, exposure times had to be increased 1.5x to 5x compared to experimental images. For histology, slides were stained with hemotoxylin and eosin (H&E), and images were obtained by light photomicroscopy using a standard SPOT CCD camera on a Zeiss Atto Arc HBO 110W upright microscope.

Results

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Macroscopic inspection of the vas deferens in the vasectomized rats revealed the presence of a granuloma at the ligature site in 4 of the 6 rats by 7 days postvasectomy. The rest of the reproductive tract appeared grossly normal. H&E staining of the vas deferens revealed histologic changes of luminal epithelium in the vicinity of the granuloma (Figure 1). The deferential epithelium in the vasectomized rats had lost the brush border present in the normal control, and the columnar epithelium had taken on an overcrowded, disorganized appearance (Figure 1A through D). An obvious inflammatory cell infiltrate was present within the lumen of the vas deferens in the vasectomized animals, in addition to immune cells infiltrating the smooth musculature of the wall of the vas deferens (Figure 1C and D). There was an apparent infiltrate surrounding the sperm granuloma (Figure 1E and F).

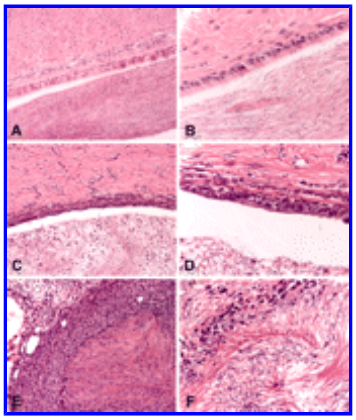

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Figure 1. Rat vas deferens tissue stained with hematoxylin and eosin. **(A)** Normal vas deferens epithelium (200x), **(B)** Normal vas deferens epithelium (400x), **(C)** Vas deferens epithelium from day 7 vasectomized rat (200x), **(D)** Vas deferens epithelium from day 7 vasectomized rat (400x), **(E)** Wall of granuloma from day 7 vasectomized rat (200x), **(F)** Granuloma from day 7 vasectomized rat (400x).

To determine if vasectomy affects the potential synthesis of epididymal secretory proteins, levels of the Crisp-1 and clusterin mRNA were determined at various times after ligating the vas deferens. The Crisp-1 gene encodes the well-known secretory proteins of the epididymis, Proteins D and E. Clusterin is a secretory protein expressed in both the testis and the epididymis (Sylvester et al, 1984). The results of the Northern blot analysis for Crisp-1 and clusterin mRNA are shown in Figure 2. No temporal changes after vasectomy were detected for either mRNA in any region of the epididymis. Consistent with previous studies, the highest levels of Crisp-1 mRNA were found in the corpus and cauda epididymis and for clusterin in the caput epididymis (Cyr and Robaire, 1992; Mattmueller and Hinton, 1992; Roberts et al, 2001).

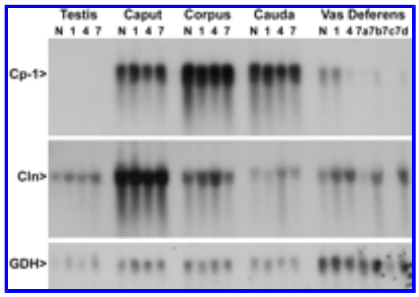

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Figure 2. Autoradiogram of Northern blots probed with a cDNA for rat Crisp-1 (Cp-1), clusterin (Cln), and GapDH (GDH) as a control. RNA samples are from normal untreated rats (N) and vasectomized rats at 1, 4, and 7 days postvasectomy (1, 4, 7). At 7 days postvasectomy, some experimental animals developed sperm granulomas. RNA isolated from the vas deferens alone, the granuloma alone, or the vas deferens and granuloma together were each analyzed by Northern blot. 7a, RNA isolated from the vas deferens of rats without granuloma. 7b, RNA isolated from the vas deferens tissue only of rats with granuloma. 7c, RNA isolated from the granuloma only. 7d, RNA isolated from vas deferens tissue and the granuloma together. The blots demonstrate the normal levels and distribution of Crisp-1 and clusterin mRNA expression in the testis and epididymis, with no apparent effect of vasectomy on mRNA levels in any tissue.

The presence of a granuloma in several of the animals indicated that an inflammatory process, likely secondary to sperm extravasation, was present in the vas deferens of these animals. Osteopontin is a known marker of inflammation, secreted by histiocytes (Carlson et al, 1997). It has also been shown to be expressed by the epididymal epithelium (Siiteri et al, 1995). When osteopontin cDNA was used to probe the Northern blots, the message was up-regulated by day 7 in the caput epididymis, compared to GAPDH controls, and markedly increased in the vas deferens (Figure 3). The increase in osteopontin mRNA in the vas deferens was severalfold higher than in the caput epididymis and appeared to be increased in both the vas deferens tissue (Figure 3, lane 7a) as well as in the granuloma (Figure 3, lane 7c).

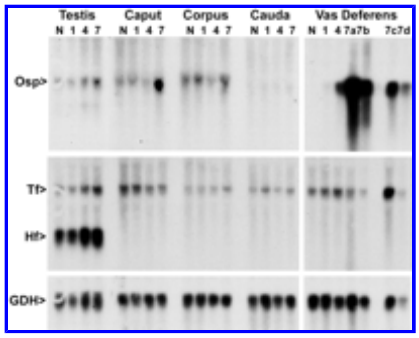

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Figure 3. Autoradiogram of Northern blots probed with a cDNA for rat osteopontin (Osn), transferrin (Tf), and GapDH (GDH) as a control. The transferrin cDNA cross-reacts with the hemiferrin (Hf) mRNA from germ cells in the testis. RNA samples are from normal untreated rats (N) and vasectomized rats at 1, 4, and 7 days postvasectomy (1, 4, 7). At 7 days postvasectomy, some experimental animals developed sperm granulomas. RNA isolated from the vas deferens alone, the granuloma alone, or the vas deferens and granuloma together were each analyzed by Northern blot. 7a, RNA isolated from the vas deferens of rats without granuloma. 7b, RNA isolated from the vas deferens tissue only of rats with granuloma. 7c, RNA isolated from the granuloma only. 7d, RNA isolated from vas deferens tissue and the granuloma together. The blots demonstrate an increase in osteopontin mRNA expression in the vas deferens with time after vasectomy as well as in the granuloma. Osteopontin mRNA is also increased at 7 days in the caput epididymis. Transferrin mRNA is also present in mRNA from the granuloma, and the levels of this mRNA appear to decrease with time after vasectomy in the caput epididymis.

Since the high expression of osteopontin mRNA in the vas deferens could be due, at least in part, to an infiltration of immune cells, the Northern blots were probed for transferrin mRNA, a message also expressed by lymphocytes (Bowman et al, 1988). The Northern blot analysis (Figure 3) showed the expected band for transferrin in the testis; a Sertoli cell message at 2.6 kb and a smaller message (0.9 kb) for germ cell hemiferrin mRNA (Stallard et al, 1991). Transferrin mRNA was also detected in the epididymis and in the vas deferens. The level of transferrin mRNA was very low and unchanged by vasectomy in the corpus and cauda epididymis. However, transferrin mRNA was easily detected in the caput epididymis and appeared to decrease with time postvasectomy. The level of transferrin mRNA in the vas deferens was very low in control animals but appeared to increase with time postvasectomy. The highest levels of transferrin mRNA were found in the granuloma tissue RNA, indicating that the primary source of transferrin in the vas deferens postvasectomy may come from infiltrating immune cells.

The levels of all 4 mRNAs used in this study were unaffected by the sham vasectomy procedure and reflected the established pattern for normal animals (Figure 4).

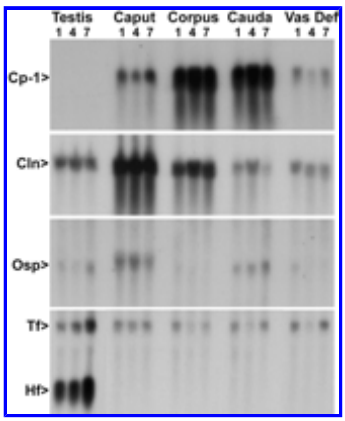

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Figure 4. Northern blot analysis of total RNA isolated probed from tissues of sham operated rats. The blots were probed with cDNAs for Crisp-1 (Cp-1), clusterin (Cln), osteopontin (Osn), and transferrin (Tf). This blot demonstrates the expected pattern of expression for each of these mRNAs and shows no increase or decrease in expression correlated to the sham operation.

Tissue sections were immunologically stained with antibodies to osteopontin (Figure 5) and transferrin (Figure 6) to determine the location of expression of these 2 proteins. The vas deferens was immunologically stained with osteopontin antibody to assess whether the protein's origin was in the inflammatory infiltrate or in the vas deferens itself. The staining pattern revealed osteopontin protein in both the vas deferens epithelium and the granuloma (Figure 5). The high fluorescence in the granuloma appeared to correspond to inflammatory cells ringing the granuloma, apparent in the H&E staining of these sections (Figure 1). On closer examination, it was apparent that the basal aspect of the caput epididymis epithelium was also labeled for osteopontin.

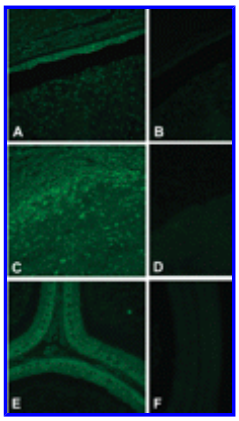

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Figure 5. Tissue sections probed with mouse monoclonal anti-rat osteopontin antibody followed by FITC-labelled anti mouse antibody **(A, C, E)** or tissues probed with secondary antibody only **(B, D, F)**. **(A, B)** Vas deferens epithelium from day 7 vasectomized rat (200x) showing specific osteopontin staining the epithelial cells of the vas deferens as well as staining of sperm in the lumen, **(C,D)** Wall of granuloma from day 7 vasectomized rat (200x) showing staining of cells within the granuloma **(E, F)** Caput epididymis from day 7 vasectomized rat (400x) showing uniform staining of the epithelial cells.

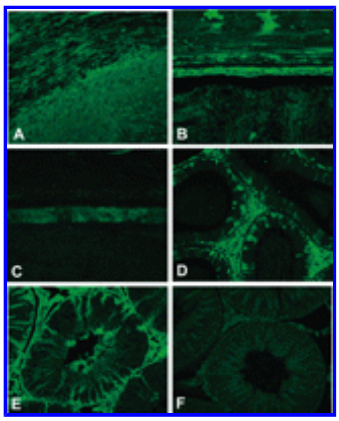

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Figure 6. Tissue sections probed with rabbit anti-rat transferrin antibody followed by FITC-labeled anti-rabbit antibody. **(A)** Wall of granuloma from day 7 vasectomized rat (200x) showing clear transferrin immunostaining throughout the granuloma, **(B)** Vas deferens epithelium from day 7 vasectomized rat (200x) showing immunostaining for transferrin in the epithelium and smooth muscle wall, **(C)** Vas deferens from a sham operated rat (200x) showing staining only within the epithelium, **(D)** Caput epididymis from a sham operated rat (200x) showing cytoplasmic staining of many epithelial cells and intense staining in the interstitial space, **(E)** Testis from a sham operated rat (200x) showing cytoplasmic staining in Sertoli cells and intense staining in the interstitial space, **(F)** Testis from a sham operated rat probed with secondary antibody only (200x) showing a complete lack of nonspecific staining.

The vas deferens of normal and vasectomized animals was also probed for the location of transferrin synthesis. The granuloma itself was intensely immunostained for transferrin, likely due in part to the infiltration of immune cells (Figure 6A). In vasectomized animals, transferrin synthesis also appeared to be significantly up-regulated in the luminal epithelium of the vas deferens (Figure 6B). This signal was greatest closest to the granuloma and decreased to normal levels further from the granuloma. In normal animals, the luminal epithelium was stained minimally (Figure 6C). Transferrin staining in the normal epididymis appeared to be most prominent in the most proximal caput,

decreasing in intensity with distance from the testis, consistent with the mRNA levels. In the caput epididymis, the localization of transferrin appeared to be in both basal and principal cells, with strong interstitial staining (Figure 6D). Transferrin staining was not detectable in the corpus or cauda (data not shown). Because transferrin expression in the epididymis has not been previously reported, we probed normal testis sections with anti-transferrin antibody as a positive control (Figure 6E). Transferrin staining was detected in the Sertoli cell cytoplasm and in the interstitial space, similar to previously reported staining patterns (Sylvester et al, 1984).

Discussion

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Vasectomy has provided a fast, cheap, and effective method of birth control for many years. With the development of microsurgery, this previously permanent procedure can now be reversed. This development has led to new interest in changes in the male reproductive tract after vasectomy and in whether or not these changes may be reversible. This study was designed to evaluate the function of the epididymis and vas deferens after vasectomy by measuring mRNA synthesis within the reproductive tract of the rat after obstruction of the vas deferens. The site of protein synthesis, corresponding to high levels of mRNA, was determined by immunocytochemistry. Two mRNAs for major epididymal secretory proteins, Crisp-1 and clusterin, were investigated, as well as mRNAs and protein expressed by inflammatory cells, osteopontin, and transferrin.

"Crisp" refers to a family of cysteine-rich secretory proteins synthesized within the male reproductive tract and salivary glands (Eberspaecher et al, 1995; Kratzschmar et al, 1996). Crisp-1, also known as acidic epididymal glycoprotein (AEG) or Protein DE, is expressed primarily in the epididymis (Lea and French, 1981; Brooks, 1982; Eberspaecher et al, 1995). The expression of Crisp-1 is under androgen control, and the Crisp-1 gene contains several consensus recognition sites for steroid hormone receptors (Schwidetzky et al, 1997; Klemme et al, 1999; Roberts et al, 2001). Crisp-1 becomes bound to the plasma membrane of sperm as they traverse the epididymis, and it has been shown to suppress capacitation in epididymal sperm (Roberts et al, 2003). Loss of this important epididymal protein would be expected to compromise sperm maturation, potentially leading to male infertility. In this study, no temporal changes in Crisp-1 mRNA levels were noted in the epididymis after vasectomy, indicating that sperm acquisition of this protein should be normal postvasectomy. This finding stands in contrast to recent studies showing that Crisp-1 protein synthesis by the caput epididymis is reduced 14 days after vasal obstruction (Turner et al, 1999). This decrease in synthesis was not reversed after vasectomy reversal (Turner et al, 2000). This apparent inconsistency may, at least in part, be explained by the different times postvasectomy that Crisp-1 measurements were taken. Additionally, it is possible that Crisp-1 secretion may decrease without a corresponding decrease in cellular Crisp-1 mRNA levels.

Clusterin (SGP-2; Apolipoprotein J) is a heavily glycosylated protein of approximately 70 000–80 000 daltons (Griswold et al, 1986). It is produced by Sertoli cells in the testis and is a major secretory protein of caput epididymis epithelial cells (Sylvester et al, 1984; Mattmueller and Hinton, 1992). Clusterin has been implicated in cell adhesion and aggregation (Silkensen et al, 1999), lipid transport (Burkey et al, 1992), and cell apoptosis (Buttyan et al, 1989). However, little or no temporal changes in clusterin mRNA levels after vasectomy were noted in this study. This is consistent with the results obtained by Turner et al (Turner et al, 1999) in their experiments with vasectomized rats. However, the lack of change in clusterin expression is somewhat unexpected, since clusterin is known to be up-regulated in tissues that have been injured or are undergoing programmed cell death (Liu et al, 1999; Yang et al, 2000). The lack of change in Crisp-1

and clusterin mRNA suggests that vasectomy does not cause acute trauma in the epididymis.

Osteopontin is a secretory protein found in numerous types of luminal epithelial cells and in vascular smooth muscle cells (for a review see Rodan, 1995). It is characterized as a cell attachment protein and has a role in modulating inflammatory responses. It is an important protein in the cascade of events leading to tissue repair after injury. Recently, it has been shown by RT-PCR and immunocytochemistry that osteopontin mRNA and protein are present in the rat testis (predominantly Sertoli cells) and epididymal epithelium and on the surface of sperm (Siiteri et al, 1995). The function of osteopontin on the sperm surface is unclear, but it may be involved in epithelial-germ cell interactions or as a calcium binding protein, preventing premature capacitation.

Osteopontin has also been implicated in granulomatous inflammation under a variety of conditions, including foreign body reactions, sarcoidosis, and rheumatoid nodule formation. The protein is expressed by histiocytes and may function in histiocyte migration and recruitment, cell adhesion, and phagocytosis (Carlson et al, 1997). In the current study, a substantial increase in osteopontin mRNA within the caput epididymis and vas deferens tissue was observed after vasectomy. From the results of the Northern blots in combination with the immunostained tissue sections, it seems that this osteopontin signal is the product of both an inflammatory reaction in the granuloma and an upregulation within the luminal epithelium of the vas deferens and the epithelium of the caput epididymis. This is consistent with osteopontin's role in inflammation and tissue injury.

Transferrin, an iron-binding protein, is expressed at very high levels in the liver but is also expressed in tissues with blood barriers, such as the brain and testis (Bowman et al, 1988). Testicular transferrin is produced by Sertoli cells, and its synthesis is influenced by hormones, paracrine factors, and germ cells (Hugly et al, 1988; Anthony et al, 1991; Roberts et al, 1991). In addition, transferrin is reportedly produced by immune cells, including lymphocytes (Bowman et al, 1988). Trends in transferrin mRNA levels varied in this study; the signal decreased with time after vasectomy in the caput epididymis but increased with time in the vas deferens and granuloma tissue. The immunocytochemical data showed the expected Sertoli cell staining in the testis. Although transferrin expression was previously thought to be testis-specific within the male reproductive tract, an apparent epithelial cell staining within the proximal caput epididymis was found. The interstitia of both the testis and epididymis were highly fluorescent due to the presence of serum transferrin. In addition, both the deferential epithelium and granuloma tissue stained for transferrin protein. This is the first report, to our knowledge, of the synthesis of transferrin in the epididymis and vas deferens. Detection of transferrin mRNA in the epididymis, with highest levels in the caput, has also been reported by gene chip analysis in the mouse epididymis (Johnston et al, 2005).

In summary, significant decreases after vasectomy in mRNAs encoding important epididymal secretory proteins were not seen within 7 days postvasectomy. However, this does not preclude the changes in epididymal function demonstrated by others at later times postvasectomy (Turner et al, 1999). While clusterin is not up-regulated by vasectomy-induced injury, osteopontin and transferrin are up-regulated within the vas deferens luminal epithelium and within infiltrating cells of the sperm granuloma postvasectomy. This study shows that acute effects of vasectomy are limited primarily to acute inflammation at the site of ligation, as evidenced by the major increase in transferrin and osteopontin expression.

Footnotes

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