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Local Cell Mediated Immune Reaction in Primary Obstructive Male Infertility

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Abstract

Increased intra-epididymal pressure due to obstruction causes a breach in the epididymal lining exposing the highly antigenic sperms to the interstitium. When the exposure is small and intermittent, it is likely to induce a local humoral response which may affect sperm maturation and also react with the epididymal epithelium producing irreversible histological changes. The present study on 30 patients of primary obstructive infertility found direct and indirect evidence of the production of antisperm antibodies locally. ELISA for antisperm antibodies was positive in the epididymal fluid in 16/30 (53%) of the patients. Indirect evidence of the role of local antigen-antibody reaction in the epididymis is apparent in 22/30 (73%) patients who had a lymphocytic infiltrate. The local presence of antisperm antibodies in the epididymal fluid correlated well with the presence of lymphocytic infiltration in the interstitium ($p < 0.05$). In our study 16/30 patients had a positive ELISA for antisperm antibody and all these patients had interstitial lymphocytic infiltration. In addition eight patients with a negative ELISA also showed interstitial lymphocytic infiltration. Thus 22/30 (73%) patients had an evidence of a local immune reaction directly in the form of a positive ELISA for antisperm antibodies in the epididymal fluid and / or indirect evidence in the form of lymphocytic infiltrate in the interstitium. None of the five controls had either a positive ELISA or lymphocytic infiltrate, this difference was found to be statistically significant ($p < 0.005$).

Keywords: Male Infertility, Autoimmunity, Testis, Epididymis, Histology

1. Introduction

The reported prevalence of antisperm antibodies (ASA) varies depending on the modality of the immunological screening. Circulating ASA detected with indirect tests ranges from 8.1% to 30.3% in unselected men with infertile marriages; at low titres they were also reported in 2.4% to 10% of fertile men [1]. When stricter criteria were used, the prevalence of ASA in men with infertile marriages was 4.7% to 7.5%. Obstruction leading to the production of antisperm antibodies is evidenced by the fact that 34% – 74 % men develop antisperm antibodies post vasectomy [2]. Though the association of epididymal occlusion with antisperm antibodies has not been widely studied, a recent report shows that antisperm antibodies are present in the serum of men with epididymal occlusion, whether of infective or congenital origin. The incidence is higher when occlusion is more distal [3].

During the onset of spermatogenesis at puberty, new developmental antigens make their appearance on the sperm surface [4]. Since immune tolerance for self-antigens is expressed neonatally, these newly appearing sperm antigens may be immunogenic. It has been theorized that sequestration of developing sperm by the blood testis barrier prevents the generation of autoantibodies to sperm [5]. Normally the epididymal epithelium is well adapted to prevent transwall migration of immunologically active material. However, in case of obstructive azoospermia, leakage of spermatozoal degradation fragments occurring proximal to the site of obstruction in the epididymis [6], or trauma to the testis or a breach of the male reproductive tract allows the sperm antigens to escape into the microcapillaries surrounding the reproductive tract and then into the systemic circulation and stimulate a humoral immune response. Genital tract infections can also induce antisperm antibodies production

due to a breach in blood testis barrier due to inflammation or by production of antibodies against the infective agents like Chlamydia which cross react with spermatozoa [7]. Approximately 34% to 74% prevalence of ASA is reported in men who have undergone a vasectomy, with their persistence in 38% to 60% following successful vasovasostomy [1] which may have a negative impact on fertility.

The deleterious effect of antisperm antibodies on fertility has been well documented [8-11]. Also, the epididymis is considered to be the most likely source of antibody secretion. It is populated with lymphocytes and macrophages [12]. The secretion of antibodies by the epididymis may be increased in cases of obstructive infertility as suggested by the presence of inflammatory cells including plasma cells, in the interstitium of these cases [13].

Increased intra-epididymal pressure due to obstruction has been reported to cause a breach in the epididymal lining exposing the highly antigenic sperms to the interstitium. When the exposure is large antisperm antibodies are produced in the serum. However, small, intermittent exposure to sperms is likely to induce a local humoral response which may not manifest itself in the serum. These antisperm antibodies secreted locally in the epididymis would act on the already disintegrating spermatozoa and further impair their function and maturation. It is possible that the antigen-antibody complexes also react with the epididymal epithelium producing irreversible histological changes, which impair the function of the epididididymis, in turn impairing sperm maturation. Thus, the persistence of infertility in patients with obstruction to the egress of sperms even after a patent anastomosis could be due to the abnormal morphology or impaired function of the spermatozoa caused by locally secreted antisperm antibodies. Further, with progress in assisted reproductive techniques, the role of ASA in male infertility is becoming better defined.

We believe that repeated exposure to small amounts of extravasated sperms in the epididymal interstitium would be sufficient to produce antisperm antibodies locally which could then initiate a local cell mediated immune reaction with its attendant effects on the epididymis and sperms. The role of local immune reaction in obstructive infertility has not been widely studied. The present study focuses on the role of local cell mediated immune response in primary obstructive male infertility.

2. Materials and Methods

Thirty patients of primary obstructive infertility with azoospermia, presence of fructose in the semen and normal spermatogenesis were included. Details of material and methods have been described in an earlier report [14]. Six patients with bilateral absence of vas deferens were also included in our study because of our policy to confirm absence of vas by scrotal exploration and in these patients to an artificial spermatocele in these patients to give them a chance for utilizing one of the cheaper methods of assisted reproduction, *i.e.* intra-uterine insemination of aspirated sperms.

The remaining patients were subjected to a single layer side-to-side vasoepididymostomy if the epididymal ducts were dilated, microscopic examination of the epididymal fluid revealed the presence of sperms and the distal vas was patent [14]. The epididymis was incised and tissue taken for histological examination. The epididymal fluid was taken with a micro-pipette was diluted with normal saline for determination of anti-sperm antibodies in the supernatant fluid with the precipitated sperms being used to study sperm morphology and other studies. The procedure was completed after taking a testicular biopsy which included part of tunica albuginea.

Five controls were also studied. They were of proven fertility. Testicular biopsy and epididymal wedge biopsy were taken at the time of autopsy or from patients who underwent orchiectomy for any reason except for testicular torsion or testicular malignancy. Epididymal fluid was also aspirated for cytology for sperm morphology and also for analysis of antisperm antibodies.

Grade of spermatogenesis in the testicular tissue was done according to the accepted criteria [15]. The interstitium was specially screened for presence of lymphocytes, plasma cells, histiocytes. Mature spermatids will be identified as oval cells with dark densely stained chromatin. The number of mature spermatids will be totaled in twenty tubules and the total be divided by twenty to give an estimate of number of mature spermatids per tubule for quantitation of mature spermatids per tubule [16].

The histologic features were recorded on a pre-determined performa as described earlier [14].

ELISA technique was used to detect antisperm antibodies by utilizing a commercially available kit. The kit is an indirect noncompetitive enzyme immunoassay for the semiquantitative and qualitative determination of antibodies directed to spermatozoa surface antigen. The wells of a microtiter plate are coated with spermatozoa surface antigen. Sperm specific antibodies present in the patient sample bind to the antigen. In a second step the antigen-antibody complex reacts with an enzyme labeled second antibody (Enzyme Conjugate) which leads to the formation of an enzyme labeled antigen-antibody sandwich complex. The enzyme label converts added substrate to form a colored solution. The rate of color formation from the chromogen is a function of enzyme

conjugate complexed with the bound antibody and thus is proportional to the initial concentration of the respective antibodies in the patient sample. The detection limit of the assay is 0.2 U/ml and permits the determination of lgG, IgM and lgA antibodies directed against sperm surface antigens.

The Chi square test was used for statistical analysis with Yates modification wherever applicable.

3. Results

The age of the patients in the study group ranged from 24-49 years (mean: 31.1 yrs.; median: 30 yrs.). Forty three percent of the patients were > 30 years old at presentation. None of the patients had any documented history of genital infections or any history suggestive of prior urinary tract infections. None had suffered from tuberculosis.

Three patients had significant levels of antisperm antibody levels in the serum. However, only one of these patients had positive ELISA for antisperm antibodies in the epididymal fluid. On the other hand, 15 out of 27 patients with absence of antisperm bodies in blood had a positive ELISA for presence of antibodies in the epidiymal fluid. This finding was not found to be statistically significant.

At operation the vas was absent in 5 patients (16.7%); 22/25 of the remaining patients had a patent vas deferens (88%). Discharge on cutting the epididymal duct was minimal (+) in 9 (30%), moderate (++) in 10 (33.3%), profuse (+++) in 5 (16.7%) and absent in 6 (20%). In the five patients with an absent vas epididymal discharge was absent in 40%, minimal in 40% and moderate in 20%.

Epididymal distension correlated well with discharge on cutting the epididymis. Thus, all the six patients with no discharge on cutting the epididymis, did not have distended epididymis although 7/13 patients without a distended epididymis still had discharge on cutting the epididymis (1 + in 6 patients and 2 + in one patient). All patients with distended epididymis had 1+ to 3 + discharge on cutting the epididymis. However, the post operative period is too short to assess the effect of these findings on the outcome of the surgery.

ELISA for antisperm antibodies in the epididymal fluid was found to be positive in 16/30 (53.3%) patients. Negative results may have occurred in the remaining patients either because of the low concentration or the small amount of epididymal fluid available which was further diluted by the saline in which it was collected. Two of the patients with significant ELISA in the epididymal fluid had positive antisperm antibodies in the serum.

The histological findings of the epididymis and their correlation with ELISA results are represented in **Table 1**.

Dilatation of ducts, epithelial breach and sperm extravasation can be considered a direct result of obstruction and was demonstrated in 63.3%, 43.3% and 26.7% of the patients under study (**Figures 1 & 2**). Sperm ingestion by macrophages was reported in 33.3% of the cases (**Figure 3**); in 8 cases epididymal discharge could be obtained and of these 75% had significant ELISA. Amongst the 22 cases with lymphocytic (majority T-lymphocytes), macrophage and plasma cell infiltration (**Figures 4 & 5**), 100% of the cases in whom epididymal fluid could be obtained had significant ELISA. In 12/14 patients with loss of cilia there was associated ductal dilatation; significantly 78.6% also had lymphocytic infiltration and in 60% the ELISA was significant.

None of the control group patients had any humoral or cellular evidence of local immune reaction.

Thus, 22/30 patients had evidence of local immune reaction in the form of interstitial inflammatory cell infiltrate in epididymal tissue and/or a positive ELISA for antiserm antibodies in epididymal fluid while 0/5 controls had evidence of local immune reaction. The difference between cases and controls was statistically significant ($p < 0.005$).

4. Discussion

Sperms are highly antigenic cells. During the onset of spermatogenesis at puberty, new developmental antigens make their appearance on the sperm surface [4]. It has been theorized that sequestration of developing sperm by the blood-testis barrier prevents the generation of autoan-

Table 1. Effect of obstruction on the epididymal histology.

Histological findings	Patients (n = 30)	Epididymal fluid absent	Significant ELISA in epididymal fluid
Dilatation of ducts	19	3	9/16
Loss of cilia	14	4	6/10
Presence of macrophages in ducts	18	4	9/14
Sperm ingestion by macrophages	10	2	6/8
Sperm extravasation	8	2	4/6
Epithelial breach	13	2	8/11
Lymphocytic and plasma cell infiltration in interstitium	22	6	16/16
Macrophage infiltration in interstitium	4	0	3/4

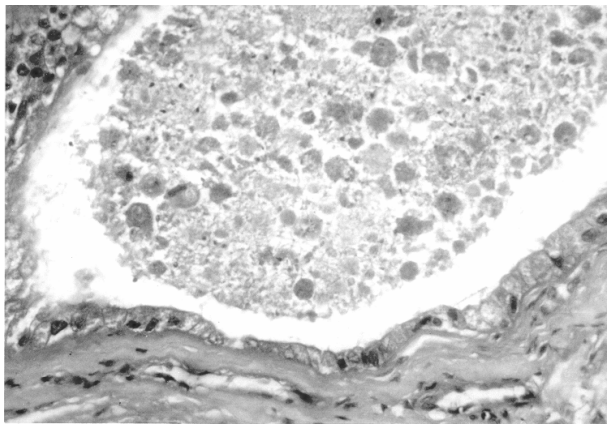

Figure 1. Photomicrograph of the epididymis showing dilated ducts with focal flattening of the epithelium (H&E x400).

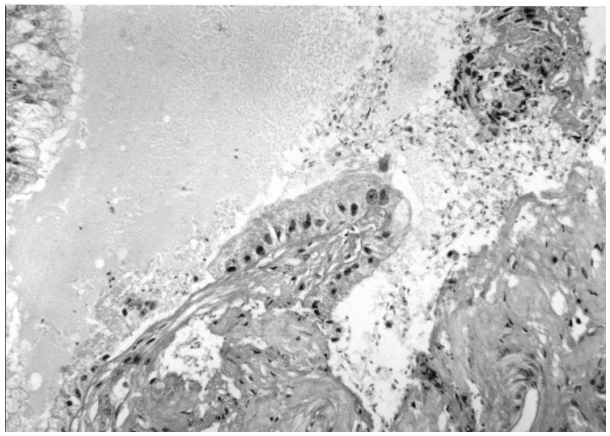

Figure 2. Photomicrograph of the epididymis showing epithelial flattening and breach, and extravasation of sperms. (H&E x400).

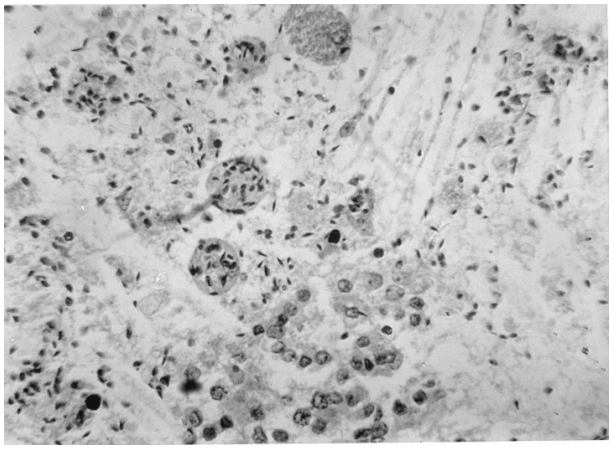

Figure 3. Photomicrograph of the contents of the dilated epididymal ducts. Macrophages with phagocytosed sperms and desquamated lining cells are seen. (H&E x400)

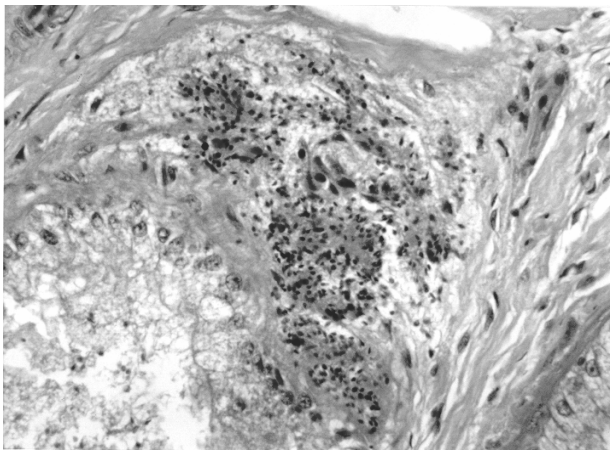

Figure 4. Photomicrograph of the epididymis showing sperm extravasation with interstitial cell infiltration (H&E x400).

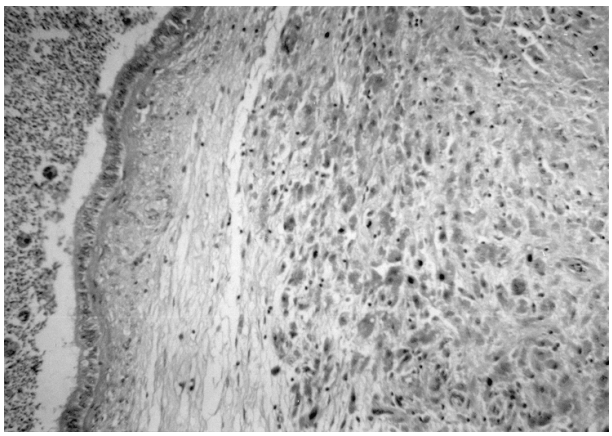

Figure 5. Photomicrograph of the epididymis showing interstitial collection of macrophages with a dilated epididymal duct to the left. (H&E x400)

tibodies to sperm [5]. The products of sperm breakdown on absorption would be an immense immunelogcal stimulus leading to immune response. However, T-lymphocytes, representing 12.7% of the mucosal cell population of the epididymal epithelium [17] act as the immunological suppressor barrier preventing such a response from occurring. Sperms are also segregated in the epididymis due to the presence of the tight junction complexes in the lining epithelium of the epididymis Which prevent the leakage of sperm antigens and prevent their contact with local and circulating immune effector cells Any breach in the reproductive tract due to the various factors leads an antibody response against the sperm. The two major classes of antisperm antibodies are IgG and IgA. IgG is a systemic immunoglobulin that is secreted in a variety of tissues and fluid compartments. IgA, on the other hand, is a secretory antibody whose production may be locally mediated. Significant levels of IgM do not reach the reproductive tract fluid in the male and

it is unlikely that IgM antibodies contribute to infertility. We studied the antisperm antibodies (IgA, IgG and IgM) in the serum of our patients and found that 3/30 patients were positive for the same. de Kretser *et al* have reported that antisperm antibodies occur more frequently in the serum of men with obstructive infertility than the normal population [3]. This finding was confirmed in our study where 3/30 (10%) of our patients had positive serum antisperm antibodies as compared to 2% in the normal fertile men. Various authors have studied the effect of obstruction on the epididymal histology and on the sperms. Rajalakshmi *et al* found degenerative changes due to pressure effects [18], while McConnel observed fibrosis, sperm granuloma and interstitial macrophages [19]. Phadke observed epithelial breach, sperm extravasation, macrophages and interstitial inflammatory cells [13]. Hargreave *et al* observed interstitial infiltration by lymphocytes, macrophages and plasma cells with fibrosis and perivasculitis [20]. Phadke also observed ingestion of sperms by macrophages [13]. Rajalakshmi *et al* observed a higher percentage of morphological abnormalities and poor motility [18]. The commonest abnormal forms were sperms with pyriform head, the others being round, tapering, long and large heads as well as abnormalities in midpiece and tail.

We believe that breach of epididymal epithelium causes extravasation of sperms into the interstitium and brings then into contact with the antibody producing cells. If the exposure is not large enough to reach the lymph nodes, antisperm antibodies will not result in the serum. However, the exposure would be sufficient to produce antisperm antibodies locally which could then initiate a local cell mediated immune reaction with its attendant effects on the epididymis and sperms.

Direct evidence for the production of antisperm antibodies locally was obtained in the present study where we found that ELISA for antisperm antibodies was positive in the epididymal fluid in 16/30 (53%) of our patients. This is an important observation establishing the role of local antigen-antibody reaction in patients of primary obstructive infertility. If taken into conjunction with the fact that only three of the patients under study had raised antisperm antibodies in the serum, out of which only two patients had a positive ELISA for antisperm antibodies in the epididymal fluid, this finding assumes further significance in establishing the importance of local production of antisperm antibodies in the epididymis. We were unable to access any reported literature on this subject.

Indirect evidence of the role of local antigen-antibody reaction in the epididymis is apparent in the observations on epididymal biopsy. We found that 22/30 (73%) patients showed a lymphocytic and plasma cell infiltrate in the interstitium which is an indirect evidence of the occurrence of an immune reaction. The local presence of antisperm antibodies in the epididymal fluid correlated well with the presence of lymphocytic and plasma cell infiltration in the interstitium ($p < 0.05$). In our study 16/30 patients had a positive ELISA for antisperm antibody and all these patients had interstitial lymphocytic and plasma cell infiltration. In addition eight patients with negative ELISA also showed interstitial lymphocytic and plasma cell infiltration. Thus 22/30 (73%) patients had an evidence of a local immune reaction directly in the form of a positive ELISA for antisperm antibodies in the epididymal fluid and/or indirect evidence in the form of lymphocytic and plasma cell infiltrate in the interstitium. None of the five controls had either a positive ELISA or lymphocytic infiltrate, this difference was found to be statistically significant ($p < 0.005$).

Thus, we found a definite evidence of the presence of a local autoimmune reaction in patients of primary obstructive infertility included in our study. It is possible that in six patients where lymphocyte and plasma cell infiltration was present but ELISA for antisperm antibody was negative and those cases with macrophage infiltration but negative ELISA, the local secretion of antibodies may have been in low concentration and was not detected by ELISA.

The local antibody response was not studied in previous reports of epididymal histology in obstructive infertility [13,18]. Phadke reported epithelial breach with sperm extravasation in patients of obstructive infertility [13]. He also found increased number of intraluminal and interstitial macrophages and suggested that these cells present the antigens to the local lymphatic sites where antibodies against spermatic antigens are produced. He suggested that it is likely that in cases of obstructive infertility some antigenic components of spermatozoa are also absorbed through intact epithelium and transferred to basal capillaries which may be responsible for development of autoantibodies against spermatozoa. Other authors also in their studies of the effect of obstruction on the epididymal histology have found an increased interstitial cell inflammatory infiltrate in cases of obstructive azoospermia which is an evidence of the presence of a local immune reaction [13,18,19], Phadke reported macrophage infiltration in the interstitium in 8/32 cases and perivascular accumulation of plasma cells in two cases [13], while a lymphocytic infiltration and fibrosis was reported by McConnel [19]. These are presumptive evidence of the presence of an antigen – antibody reaction although a direct evidence of the presence local antibodies in these cases was not obtained. Since the histologic findings of the epididymis in their reports are similar to those in the present study, it is possible that

they also resulted from a local autoimmune reaction. However, lack of direct evidence of the presence of antisperm antibodies in the epididymal fluid precludes from coming to any positive conclusion regarding this aspect. In our study we found intraluminal macrophages in 18/30 cases and 9/18 of these cases had a positive ELISA for antisperm antibodies. Although this finding is statistically insignificant, the negative ELISA could be explained by the presence of antibodies in low titres in these cases.

Thus there is enough evidence to suggest that a cell mediated immune response occurs in males with primary obstructive infertility and produces changes in both the luminal and extraluminal portions of the epididymis. The local immune response is likely to impair fertility through various likely mechanisms. In most epidemiologic studies there is little evidence that suggests a cause/effect relationship between ASA and abnormality of the principal semen parameters (sperm count, motility and morphology) between infertile patients with and without ASA [1]. Sperm injury mediated by complement mediated sperm cytotoxicity is potentially possible. Although anticomplementary activity has been reported in human semen, this mechanism has been documented in the female genital tract [1,21]. Antisperm antibodies and complement deposition resulted in a dramatic loss of sperm motility, as well as in activation and aggregation (rosetting) of polymorphonuclear leukocytes (PMN) to antibody- and complement-bound sperm [1]. Several studies suggest that ASA can interfere with sperm functions involved in the fertilization process by blocking sperm-egg interactions. This would affect natural fertilization and even in assisted reproductive techniques it has to be taken into account both as an interfering factor blocking sperm-egg interactions and also as a complicating factor due to ASA production after insemination [21].

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