The American Journal of PATHOLOGY

SEPTEMBER 1970 • Volume 60, Number 3

The Immunopathology of Experimental Allergic Orchitis

Kenneth S. K. Tung, MB, BS,* Emil R. Unanue, MD† and Frank J. Dixon, MD

EXPERIMENTAL ALLERGIC ORCHITIS (EAO) is an organ-specific, immunologic disease produced in several species of animals by the injection of homologous sperm antigen mixed in complete Freund's adjuvant. The immunized animals, usually guinea pigs, make antibody,[1,2] exhibit cutaneous delayed hypersensitivity [2] and become aspermatogenic.[1,2] The pathogenesis of EAO is unclear. Freund *et al* [1,3] concluded that the main lesion was specific and selective damage of germinal cells. Inflammatory infiltrates in the interstitium were not a consistent findings in these studies. Brown *et al*,[2,4] using an antigenic preparation (testicular autoclavate) different from that used by Freund *et al*, reported early infiltrates of both polymorphonuclear neutrophils (PMNs) and mononuclear cells, principally along the ductus efferentes, the caput epididymis and the rete testis. After this early lesion, degenerative changes in the germinal cells were observed. Furthermore, by immunofluorescence, Brown *et al* demonstrated guinea pig globulin on sperm cells in seminiferous tubules soon after immunization. In contrast to the above studies, Waksman [5] reported an early lesion con-

From the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

Supported by Grant AI 07007 from the US Public Health Service and Contract AT(04-3)-410 from the Atomic Energy Commission.

Accepted for publication May 25, 1970.

Address for reprint requests: Order Publication 412, from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif 92037.

* Supported by Training Grant 5T1GM683 from the US Public Health Service. Present address: Department of Pathology, St. Louis University, St. Louis, Mo.

† Supported by a Dernham Senior Fellowship of the American Cancer Society, California Division.

sisting of mononuclear infiltrates in the testicular interstitium; direct invasion of the seminiferous tubules by these cells followed, accompanied by destruction of the germinal epithelium.

This report presents a reevaluation of the early stages of EAO in the guinea pig, using a variety of testicular antigens. Herein, aspermatogenesis was invariably preceded by transitory infiltrates of monocytes and lymphocytes around the seminiferous tubules. In contrast to the seminiferous tubules, the sperm passage* exhibited acute inflammatory reactions. Immunoglobulin G (IgG) was found to be bound exclusively to sperm in the passage.

Materials and Methods

Animals. Outbred male albino guinea pigs, weighing 500–600 g, were obtained from local suppliers; histocompatible Wright Strain 13 guinea pigs were obtained from Crest Caviary, Encinitas, California, and Horton's Laboratory Animals, Los Gatos, California.

Antigens. Four testicular antigens were used. The first three were prepared from frozen testes of outbred guinea pigs (Pel Freez Biologicals, Rogers, Ark): (1) ammonium sulfate precipitable material (ASPM), prepared according to the method of Freund *et al*;[3] (2) testicular homogenate; decapsulated testes were homogenized in phosphate-buffered saline at w/v ratio of 1:1 and the suspension was lyophilized; (3) testicular autoclavate; prepared according to Brown *et al*;[2] and (4) fresh sperm; obtained from the epididymis.

In control experiments, bovine serum albumin (BSA) (Pentex Labs, Kankakee, Illinois) was used as antigen.

Immunization of Guinea Pigs. Outbred guinea pigs were immunized with a single dose of antigen in complete Freund's adjuvant (CFA). Two milligrams of ASPM, testicular autoclavate or BSA, 10 mg of testicular homogenate or 10^8 sperm in 0.5 ml of phosphate-buffered saline were emulsified in 0.5 ml of Bayol F–Arlacel C (8.5:1.5 v/v) mixture containing 4 mg of dried *Mycobacterium tuberculosis* (strain H37 Ra, Difco Laboratories, Detroit, Mich). The mixture was injected into one hind foot pad and among 4 intradermal sites on the subject's back. Strain 13 guinea pigs were immunized with 10 mg of ASPM or 20 mg of testicular homogenate.

The control animals consisted of outbred guinea pigs that either were not immunized or were immunized with BSA in CFA, BSA in incomplete Freund's adjuvant or CFA alone. These immunized guinea pigs were examined 12–25 days after immunization.

Histologic procedure. The whole testis was fixed in Zenker's fixative for 12–24 hr after which 6 or 7 serial transverse blocks, 5 mm thick, were cut. These blocks were fixed for 12 more hr, embedded in paraffin, sectioned and stained with periodic acid-Schiff hematoxylin.

Immunofluorescent studies. The whole testis was snap-frozen in liquid nitrogen and sectioned at 4 μ in a cryostat. Smears of cells from testicular homogenates were air-dried. The tissue section or smear was processed for immunofluorescence using either the direct or indirect procedure. Testing for specificity consisted of determining whether there was reduction of fluorescence either by prior application

* In this paper, sperm passage as an anatomic entity includes the straight tubule, the rete testis, the ductus efferentes, the epididymis and the vas.

to the tissue or cells of an unconjugated specific antiserum or absorption of the fluoresceinated antiserum with its specific antigen.

Antiserums consisted of sheep and rabbit anti-guinea pig IgG, sheep anti-rabbit IgG, rabbit anti-guinea pig β1C globulin (the third component of complement, C3) and rabbit anti-BSA. Guinea pig or rabbit IgG was obtained by DEAE-cellulose column chromatography of serum and consisted of the material eluted in 0.01 M phosphate buffer at pH 7.4. C3 was prepared by the method of Mardiney and Müller-Eberhard.[6] All antiserums were obtained by 2–8 immunizations 2 weeks apart, with the antigens mixed in CFA. The antiserums, harvested 4 weeks after the last immunizing dose, were monospecific by immunoelectrophoresis. The IgG fraction of the antisera, obtained by DEAE-cellulose chromatography, was conjugated to fluorescein isothiocyanate[7] and then chromatographed on DEAE cellulose by stepwise salt concentration gradients of 0.01, 0.05 and 0.10 M phosphate buffered saline.[8] The fraction eluted with 0.05 M phosphate buffer was used in this study.

Electron Microscopy. The testes for ultrastructural studies were fixed *in situ* by arterial perfusion *in vivo* with 1% glutaraldehyde in cacodylate buffer. Under ether anesthesia, the animal's thoracic aorta was cannulated with a polyethylene tubing (Size 50, Intramedic, Clay Adams, Inc.), the tip of which was advanced to the level of the renal arteries. Through an abdominal incision, the renal arteries and the abdominal aorta inferior to the origin of the testicular arteries were clamped and the inferior vena cava above the diaphragm was cut. Each animal was rapidly perfused with about 200 ml of the fixative. Small blocks of tissue from regions of the rete testis and the seminiferous tubule were removed with the aid of a dissecting microscope and were placed in the same fixative for 2 more hrs. Tissue blocks were embedded in vestopal. Thin sections of approximately 900 Å thickness were stained with osmium tetroxide and lead citrate and viewed with a Hatachi 11A electron microscope.

Results

Immunohistologic Analysis of Testes from Control Animals

Degenerative and desquamative germinal epithelial cells were found in occasional seminiferous tubules in about one fourth of 21 untreated guinea pigs as well as one fourth of 12 guinea pigs immunized with BSA in incomplete Freund's adjuvant. These few abnormal seminiferous tubules were present always in the subcapsular region surrounding the exit of the pampiniform venous plexus. This focal change in seminiferous tubules occurred in about half of the 34 guinea pigs that were immunized with CFA (with or without BSA). Inflammatory cellular infiltrate was absent in these testes. There was no localization of IgG in the sperm cells by immunofluorescence.

Immunologic and Morphologic Analysis of Testes from Guinea Pigs Immunized with ASPM Antigen

One hundred outbred guinea pigs were immunized with ASPM in complete Freund's adjuvant and were sacrificed from 5 to 55 days after immunization; usually, one testis was studied histologically and the

other immunohistochemically. The immunopathologic findings in the seminiferous tubules and in the sperm passages were different and are described separately.

Pathology of the Seminiferous Tubules (Table 1)

HISTOPATHOLOGIC FINDINGS. Eleven testes examined between 5 and 9 days after immunization were normal. Of the 89 animals studied on or beyond the eleventh day, 69 (77%) developed disease in the seminiferous tubules.

The pathologic picture varied depending upon the time of examination of the testes after immunization (Table 1). Most of the testes examined between 11 and 21 days exhibited mononuclear cellular infiltrates. The infiltrating cells consisted predominantly of monocytes and lymphocytes dispersed among seminiferous tubules or abutting against tubular walls. Often the cells formed a nodule that appeared to protrude towards the tubular lumen. In other instances, there was a loss of integrity of the wall of the tubules, and the infiltrating cells were seen to enter the lumen (Fig 1). The mononuclear cellular infiltration could be focal, involving only a few tubules, or diffuse and extensive. In many testes examined early, the mononuclear cells surrounded normal seminiferous tubules. In testes examined later, the cells surrounded seminiferous tubules that exhibited degeneration and loss of germinal epithelium. PMNs were often prominent in severe lesions, and were seen either in the interstitium or inside the tubular lumen.

Most of the testes examined after 3 weeks exhibited aspermatogenic and atrophic tubules surrounded by scanty inflammatory cells. Some

Table 1. Pathology of Seminiferous Tubules After Immunization with ASPM and CFA

Day after immunization	Diseased testes/total examined (%)	Patterns of testicular lesion*		
		Inflammatory	Mixed	Atrophic
5–9	0/11 (0)	0	0	0
11–13	30/40 (75)	17	13	0
15–17	17†/20 (85)	5	6	4
18–26	19/24 (80)	4	4	11
29–55	5/5 (100)	0	0	5

* Inflammatory pattern denotes predominantly mononuclear infiltrates in interstitium with minimal degenerative change in the germinal epithelium. Mixed pattern denotes both mononuclear infiltrates in interstitium and tubular damage. Atrophic pattern denotes predominance of aspermatogenic tubules with few mononuclear infiltrates.

† 2 guinea pigs in this group had lesions in the sperm passage without lesions in seminiferous tubules.

evidence of regeneration of spermatocytes and spermatids appeared in the 2 testes that were examined 41 and 50 days after immunization.

The sequence of histopathologic events was studied in 20 additional guinea pigs, 14 immunized with ASPM and 6 with testicular homogenate. In each of these guinea pigs, one testis (obtained by unilateral orchiectomy) was examined 12 days after immunization and the remaining testis was obtained at sacrifice some days later. In 18 animals (Table 2), the lesion seen at 12 days consisted of focal or diffuse mononuclear cellular infiltrates that surrounded predominantly normal seminiferous tubules. In 6 of these animals the second testis showed progressive loss of germinal epithelium with a variable number of infiltrating mononuclear cells (Fig 2). In the remaining 12, however, the second testes were mainly aspermatogenic and atrophic with few mononuclear cells. It was apparent that in the course of about 10 days the pathologic picture could change from one of predominantly mononuclear cellular infiltration to one of predominantly tubular degeneration.

ULTRASTRUCTURAL STUDY. The boundary tissue of a normal seminiferous tubule is illustrated in Fig 3 and 4. Sertoli cells rest on a basal lamina that consists of two thin basement membranes and variable amounts of intervening collagen fibers. A layer of myoid cells, characterized by their ribbon-like appearance and their cytoplasmic fibrils, lies external to the basal lamina. Between the layer of myoid cells and the interstitial capillaries is a channel bound by cells with attentuated cytoplasm. These channels are believed to represent afferent lymphatics in the interstitium.[9,10]

Five diseased testes were examined 15 days after immunization with ASPM. Here monocytes and lymphocytes were most frequently encountered among infiltrating cells in the interstitial spaces. The 127 cells examined, were 50% monocytes, 25% mature lymphocytes and 12% lymphoblasts (*ie,* large lymphocytes that contained numerous cytoplasmic polyribosomes). The remainder consisted of PMNs (5%), plasma cells (3%), and cells that did not have distinctive morphologic features for classification (5%).

In many lesions the tubular basement membrane was the only structure that separated the infiltrating cells from sperm cells within the seminiferous tubules (Fig 5). In these lesions the monocytes established intimate contact with the basement membrane. Occasionally, transit of monocytes through defects in the basement membrane was observed (Fig 6).

IMMUNOFLUORESCENT STUDY. Guinea pig IgG or C3 was not detected in the cells of the seminiferous tubules of 11 guinea pigs sacri-

Table 2. Sequential Histopathologic Findings in the Two Testes of Each of 20 Guinea Pigs Immunized with Testicular Antigens in Complete Freund's Adjuvant

Guinea pig No.	Strain	Testicular antigen	Days after immunization	Patterns of Testicular Lesion*			Passage lesion
				Inflammatory	Mixed	Atrophic	
1	Albino	ASPM	12	+	—	—	+
			17	+	+	++	++
2	13	ASPM	12	+	—	—	—
			24	+	+	+	—
3	13	ASPM	12	+	—	—	—
			24	+	+	++	—
4	13	Homogenate	12	+	—	—	—
			23	—	+	—	—
5	13	Homogenate	12	+	—	—	—
			23	—	+	+	+
6	Albino	ASPM	12	+	+	—	—
			17	+	++	++	++
7	Albino	ASPM	12	+	++	++	++
			21	—	+	++	—
8	13	ASPM	12	++	+	—	—
			24	+	++	++	—
9	13	ASPM	12	+	+	—	—
			24	—	+	+	—
10	13	ASPM	12	+	+	—	—
			24	—	++	+	++
11	13	Homogenate	12	+	+	—	—
			23	—	+	++	—
12	13	Homogenate	12	+	+	—	—
			23	—	+	++	—
13	Albino	ASPM	12	++	+	—	++
			43	—	—	+	—
14	Albino	ASPM	12	+	—	—	+
			22	—	—	+	++
15	Albino	ASPM	12	+	++	++	—
			53	—	—	+	—
16	13	ASPM	12	+	—	—	—
			24	—	—	+	—
17	13	Homogenate	12	+	++	+	—
			23	—	—	+	—
18	13	Homogenate	12	+	+	—	+
			23	—	—	+	—
19	Albino	ASPM	12	—	—	—	—
			22	+	++	+	+
20	Albino	ASPM	12	—	—	—	—
			22	—	+	+	—

* Patterns as described in Table 1.
— indicates absent.
+ and ++ denote increasing severity of lesions.

ficed between 5 and 9 days after immunization, before development of morphologic lesions, and 42 guinea pigs sacrificed from 12 to 45 days after immunization, at which time disease had developed.

To increase the sensitivity of detection of IgG, several testes were also studied by indirect immunofluorescence using nonfluoresceinated rabbit anti-guinea pig IgG, followed by fluorescein-labeled sheep anti-rabbit IgG. Again, no IgG was detected in sperm cells. Smears of cell suspensions from some diseased testes also failed to disclose cell-bound IgG.

Many cells in the testes showed nonspecific fluorescence. These included PMNs, basophils, mast cells, intraluminal giant cells and round (residual) bodies situated at the basal portion of seminiferous tubules. The fluorescence of these cells was not abolished by absorption of the antiserum with the corresponding antigen. Moreover, these cells were stained positively with nonspecific conjugates such as rabbit anti-BSA. Rarely were plasma cells with cytoplasmic IgG found in testes with orchitis.

Pathology of the Sperm Passage

HISTOPATHOLOGIC FINDINGS. The lesion in the sperm passage was characterized by an acute inflammatory exudate with PMNs as the major infiltrating cells. In early lesions, PMNs were seen as a single row along the basement membrane of the ducts. In later lesions, the PMNs infiltrated the wall of the ducts, entered the lumen (Fig 7) and eventually formed an abscess. In these severe lesions a periductal accumulation of monocytes and lymphocytes was also found. The sequential histopathologic study of both testes of the same guinea pig (Table 2) suggested that a large suppurative lesion in the epididymis might resolve in 9 days; pathologic changes in the epididymis of the second testis consisted of small empty ducts and focal interstitial fibrosis.

Lesions were found in the straight tubules, rete testis and the extratesticular sperm passage, which includes ductus efferentes, epididymis and vas deferens. Among guinea pigs with lesions in the seminiferous tubules, 62% exhibited lesions in the rete testis and 47% in the extratesticular passage. Only 2 guinea pigs had lesions in the passage without involvement of the tubules (Table 1). Either the lesions were found throughout the extratesticular passage (8 of 34) or they were restricted to the ductus efferente (7 of 34) or the cauda epididymis (19 of 34).

The evolution of the passage lesions paralleled that of the tubular lesions. Both started, reached their highest incidence and resolved at about the same time.

ULTRASTRUCTURAL STUDY. The anatomy of the boundary tissue of the sperm passage and its relation to blood vessels are different from those of the seminiferous tubules. In the straight tubules, the rete testis, ductus efferente and the cauda epididymis, the epithelial cells rest on a single basement membrane that is surrounded incompletely by fibroblast-like cells and collagen bundles (Fig 4). The boundary tissues possess no myoid cells and, furthermore, the ducts are not necessarily separated from blood vessels by lymphatic channels.

Ultrastructural study of diseased sperm passages disclosed no significant findings additional to those revealed by light microscopy.

IMMUNOFLUORESCENT STUDY. Guinea pig IgG and C3 were found bound to acrosomes of sperm in the straight tubules, the rete testis and occasionally in the cauda epididymis in 13 of 42 guinea pigs studied between 11 and 25 days after immunization. In many instances, the acrosomes with bound IgG appeared agglutinated and distorted (Fig 8). Of these 13, eight had lesions in seminiferous tubules and only 2 had lesions in the rete testis. Of the 29 guinea pigs that had no detectable IgG or C3, 24 had lesions in the seminiferous tubules and 11 had lesions in the rete testis.

Hence, acrosomes with bound IgG and C3 were found more often in lumens of rete testis that were free of inflammatory infiltrates. It was difficult to determine whether a passage with acute inflammation contained sperm cells with bound IgG, however, because of the interference by nonspecific fluorescence of PMNs.

Guinea Pigs Immunized with Testicular Antigenic Preparations Other than ASPM

Guinea pigs were immunized with three other antigens: testicular homogenate, autoclavate and sperm cells. Fifteen of 16 animals immunized with testicular homogenate developed disease when examined between 11 and 14 days after immunization. None of the 4 examined 6 days after immunization showed pathology. The histopathologic and immunofluorescence findings in the seminiferous tubules were similar to those observed in guinea pigs immunized with ASPM. However, only 1 of these guinea pigs exhibited lesions in the sperm passage.

Seventeen guinea pigs immunized with autoclavate were studied 6, 11 and 15 days after immunization. Five exhibited lesions. In 3 of these, the lesion was an acute inflammatory reaction in the ductus efferentes; one was present on Day 6, the other two on Day 11 after immunization. The remaining 2 had peritubular infiltration of mononuclear cells exclusively. We could not detect sperm with bound IgG in seminiferous tubules or sperm passage in any of these animals.

All 6 guinea pigs immunized with sperm developed orchitis (between 11 and 26 days); 5 developed acute inflammatory lesions in the sperm passage. The lesions were indistinguishable from those observed in guinea pigs immunized with ASPM.

Discussion

Two types of pathologic lesions were observed in the testes of guinea pigs immunized with sperm antigens. One lesion was characterized by a transitory infiltration of mononuclear cells surrounding mainly the seminiferous tubules; the second was characterized by an accumulation of PMNs and was chiefly observed in the collecting and passage system. These two distinctly different lesions suggest that each is produced by a different mechanism. The mononuclear lesion of the seminferous tubules mimics the histopathology of delayed hypersensitivity reactions and is, indeed, most likely associated with cell-mediated immunity. As far as we could determine, this lesion was neither preceded by neutrophil infiltration, nor was it associated with IgG bound to the sperm cells of the tubules. In contrast, some of the lesions along the passage were most likely related to the local fixation of antibody followed by complement activation and chemotaxis of PMNs. In fact, IgG and C3 were found bound to sperm exclusively in the passage system. Two points are worthy of emphasis since they may explain some of the discrepant results in the literature. First, it was apparent that the peritubular infiltration with mononuclear cells often could be transitory or focal. The majority of animals studied beyond 21 days after immunization had atrophic tubules with few infiltrating cells. Yet all guinea pigs that developed aspermatogenesis had exhibited earlier the peritubular infiltrates with mononuclear cells. The transitory and focal nature of the mononuclear infiltrate may explain, therefore, why some investigators in the past have failed to observe the phenomenon consistently and consequently to give it serious consideration.[1–4] Second, different antigens produced somewhat different pathologic pictures. Thus, ASPM and sperm induced identical lesions insofar as type and severity; testicular homogenates produced extensive mononuclear cell lesions in the seminiferous tubules with little acute inflammatory reactions in the passage; testicular autoclavate, in agreement with other studies,[4] induced early acute inflammatory lesions mainly along the ductus efferentes with no great involvement of the seminiferous tubules. The reason for these differences may become apparent when the aspermatogenic antigens are better characterized physicochemically and immunologically.

At all times, Ig was found to localize exclusively to the sperm cells that were lodged in the passage system. The mechanisms for a preferential localization of antisperm antibody in the passage are under present consideration. Brown and Glynn [4] postulated that sensitized lymphoid cells present at the ductus efferentes might cause an increase in permeability at this site and permit entry of serum antibody. An alternative explanation is that guinea pigs may normally exhibit a difference in permeability and/or concentration of antibody among different regions of the testes; relative to the seminiferous tubules, the rete testis or the straight tubules may be more permeable to serum proteins. The fact that, ultrastructurally, tissues around the rete testis or the straight tubules are less complex than those in the seminiferous tubules and, in particular, lack the myoid cell layer and the lymphatic channel, is consistent with the concept of differences in permeability. The results of experiments [11] in which guinea pigs were immunized with ASPM in incomplete Freund's adjuvant disagree with the hypothesis that cell-mediated immunity is essential for antibody to enter the sperm cell compartments. These guinea pigs developed serum antibody but no delayed hypersensitivity reaction *in vitro* and no testicular pathology. The testes of these animals exhibited IgG on sperm in the rete testis and the straight tubules, but not in the seminiferous tubules. Furthermore, in other experiments we have injected ^{125}I-labeled normal IgG or IgG with antisperm antibody under the tunica albuginea of normal testes; both were found to enter the rete testis and the straight tubule, but did not cross the seminiferous tubule.[12] Other investigators have found IgG and C3, albeit in small amount, in the fluid obtained from cannulated rete testis of normal rams.[13] Taken together, these results suggest that when antisperm antibody reaches a sufficiently high concentration, it may enter the sperm cell compartment preferentially at the rete testis or the straight tubules and from these sites diffuse to other parts of the sperm passage.

The sequential study suggests that the mononuclear cell infiltrates may cause the derangement of the germinal epithelium and do not represent a nonspecific inflammatory response. Furthermore, we have now been able to reproduce similar lesions in the seminiferous tubules by the injection of syngeneic sensitized lymphocytes into the testes of Strain 13 guinea pigs.[12] The role of antibody in producing aspermatogenesis may not be important since the seminiferous tubules may not be permeable to IgG. On the other hand, antibody binding with mature sperm cells in the passage may stop them from reaching their final destination by killing, opsonizing or "blindfolding" them.

Summary and Conclusions

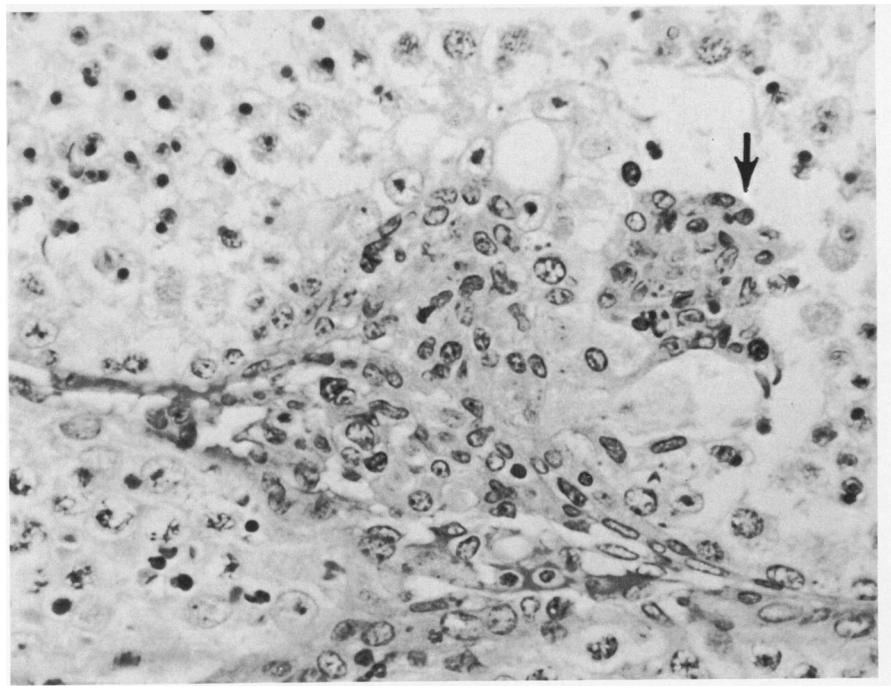
Two different types of lesions were found in testes of guinea pigs immunized to testicular antigens. One lesion, in the seminiferous tubules, was characterized by peritubular accumulation of mononuclear cellular infiltrates, followed by degeneration and loss of the germinal epithelium. There was no detectable IgG or C3 in sperm cells of the seminiferous tubules. The other lesion was present in the sperm passage and characterized by neutrophil infiltration. IgG and C3 were occasionally found on sperm acrosomes. The incidence and distribution of lesions depended upon the testicular antigen that was used for immunization. These studies suggest that local anatomic and physiologic factors may influence the distribution and character of immunopathologic lesions in this disease.

References

1. Freund J, Lipton MM, Thompson GE: Aspermatogenesis in the guinea pig induced by testicular tissue and adjuvants. J Exp Med 97:711–725, 1953
2. Brown PC, Glynn LE, Holborow EJ: The pathogenesis of experimental allergic orchitis in guinea-pigs. J Path Bact 86:505–520, 1963
3. Freund J, Thompson GE, Lipton MM: Aspermatogenesis, anaphylaxis and cutaneous sensitization induced in the guinea pig by homologous testicular extract. J Exp Med 101:591–604, 1955
4. Brown PC, Glynn LE: The early lesion of experimental allergic orchitis in guinea-pigs: an immunological correlation. J Path 98:277–282, 1969
5. Waksman BH: A histologic study of the auto-allergic testis lesion in the guinea pig. J Exp Med 109:311–324, 1959
6. Mardiney MR, Müller-Eberhard HJ: Mouse β1C globulin:production of antiserum and characterization in the complement reaction. J Immun 94:877–882, 1965
7. Clark HF, Shepard CC: A dialysis technique for preparing fluorescent antibody. Virology 20:642–644, 1963
8. Wood BT, Thompson SH, Goldstein G: Fluorescent antibody staining. III. Preparation of fluorescein-isothiocyanate-labeled antibodies. J Immun 95:225–229, 1965
9. Leak LV, Burke JF: Fine structure of the lymphatic capillary and the adjoining connective tissue area. Amer J Anat 118:785–810, 1966
10. Fawcett DW, Heidger PM, Leak LV: Lymph vascular system of the interstitial tissue of the testis as revealed by electron microscopy. J Reprod Fert 19:109–119, 1969
11. Tung KSK, Unanue ER, Dixon FJ: Unpublished data
12. Tung KSK, Unanue ER: Pathogenesis of allergic orchitis. Fed Proc 29:287, 1970
13. Johnson MH, Setchell BP: Protein and immunoglobulin content of rete testis fluid of rams. J Reprod Fert 17:403–406, 1968

We wish to thank Dr. J. D. Feldman for his advice in the electron microscopic study. Excellent technical assistance of Mr. Gerry Sandford, Mrs. Helen Hays, Miss Jean Busch and Miss Rosemarie Peña is appreciated.

[*Illustrations follow*]



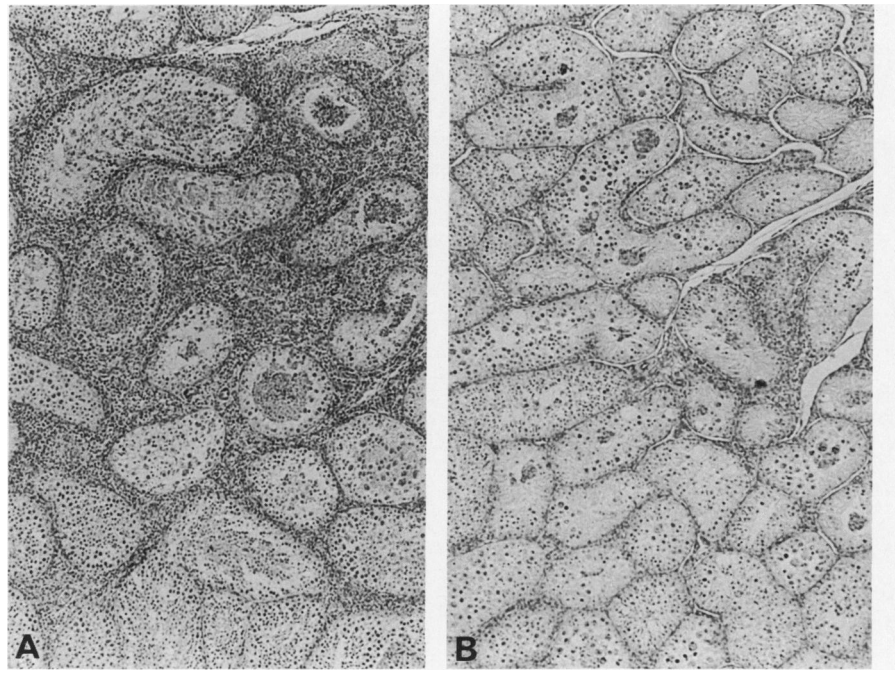


Fig 1. Photomicrograph showing focal infiltrates of lymphocytes and monocytes that protrude toward lumen of a seminiferous tubule. *Arrow* points to cluster of cells inside tubular lumen. This typical lesion can be seen focally in early disease. Testis was taken from guinea pig immunized 11 days before with ASPM in CFA. Periodic acid-Schiff hematoxylin stain. × 500.

Fig 2. Photomicrograph of representative areas of two testes from Guinea Pig 1 of Table 2. **A** was studied on Day 12 and **B** on Day 17 after immunization with ASPM in CFA. Compared with **A, B** has fewer infiltrating mononuclear cells and more tubules with degenerative changes. Periodic acid-Schiff hematoxylin stain. × 25.

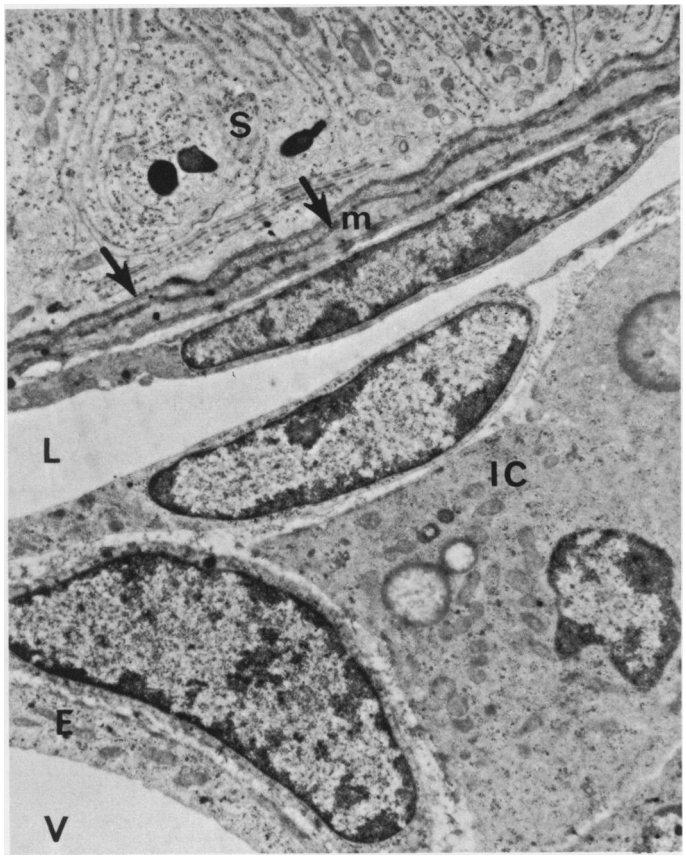


Fig 3. Electron micrograph showing the boundary tissues of seminiferous tubule of a normal guinea pig. The Sertoli cell (*S*) is separated from the lumen of a capillary (*V*) by two basement membranes (*arrows*), a myoid cell layer (*m*), a lymphatic channel (*L*), and endothelial cell (*E*) of the capillary. *IC* is an interstitial cell. Approx × 14,000.

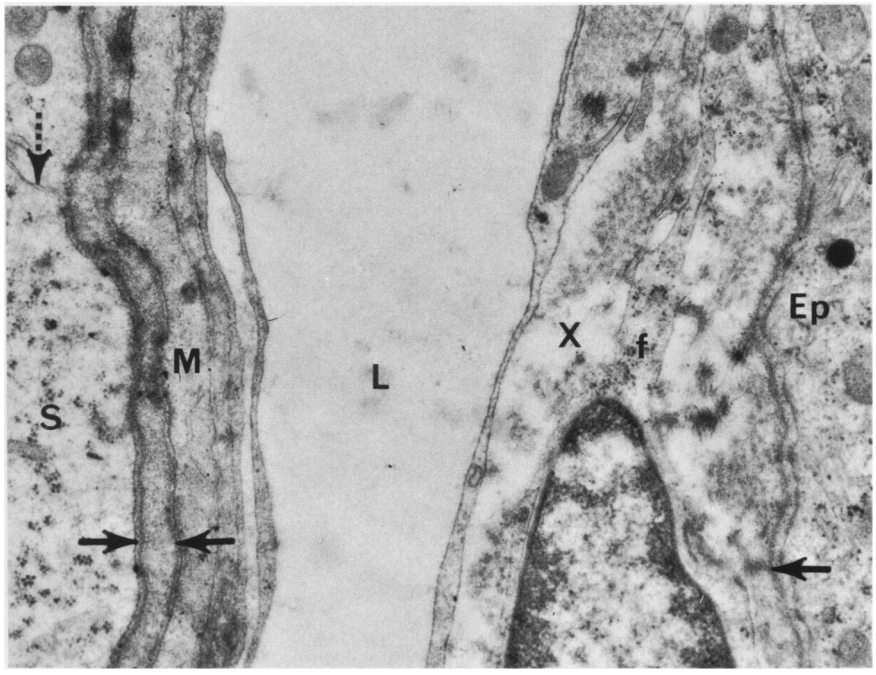


Fig 4. This electron micrograph compares boundary tissues between seminiferous tubule (*left*) and straight tubule (*right*) of normal guinea pig. *S* indicates Sertoli cell; *solid arrow(s)*, basement membrane; *broken arrow*, junction between Sertoli cells; *m*, myoid cell; *L*, lymphatic space; *f*, fibroblast-like cell; *Ep*, epithelial cell; *X*, interstitial space. Approx × 30,000.

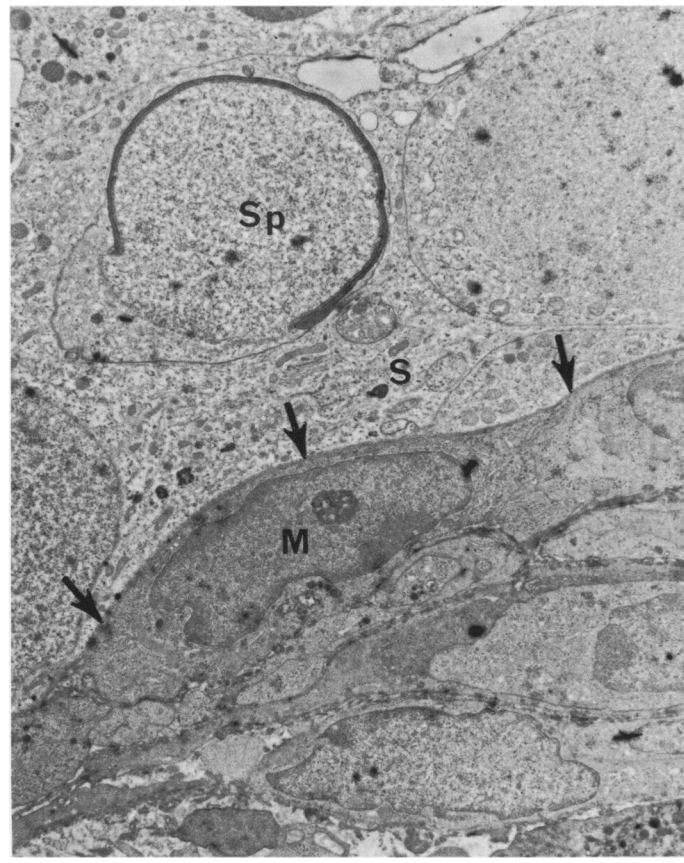


Fig 5. This electron micrograph shows infiltrating mononuclear cells abutting against seminiferous tubules. Infiltrating cells are separated from Sertoli cells (*S*) by tubular basement membrane (*arrows*). Most infiltrating cells are monocytes (*M*). Normal spermatid (*Sp*) is noted. Testis is taken from guinea pig 15 days after immunization with ASPM in CFA. Approx × 8000.

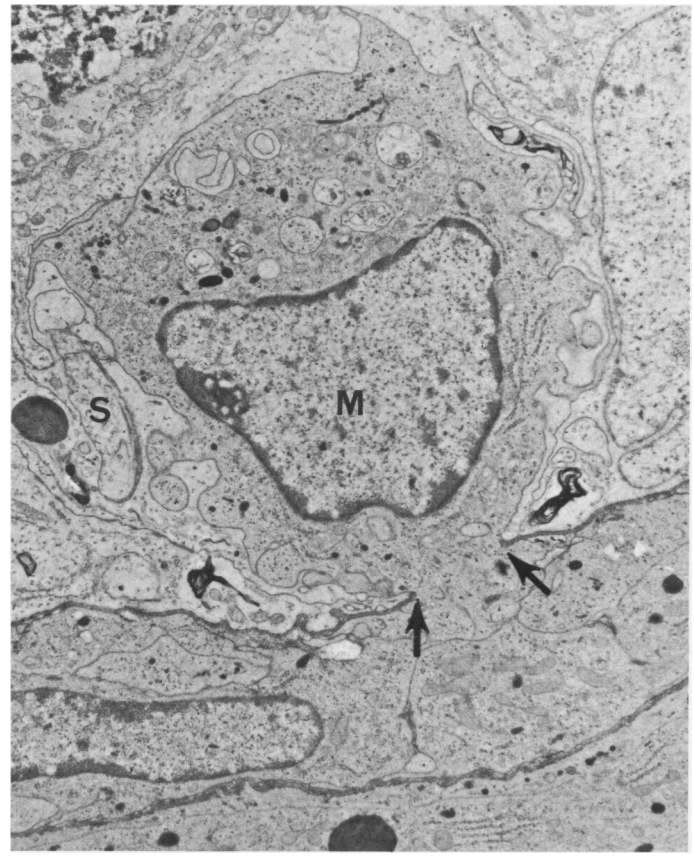


Fig 6. Electron micrograph showing transit of monocyte (*M*) through defect (*arrows*) in tubular basement membrane to establish close contact with Sertoli cells (*S*) inside tubule. Testis is taken from guinea pig 15 days after immunization with ASPM in CFA. Approx × 16,000.

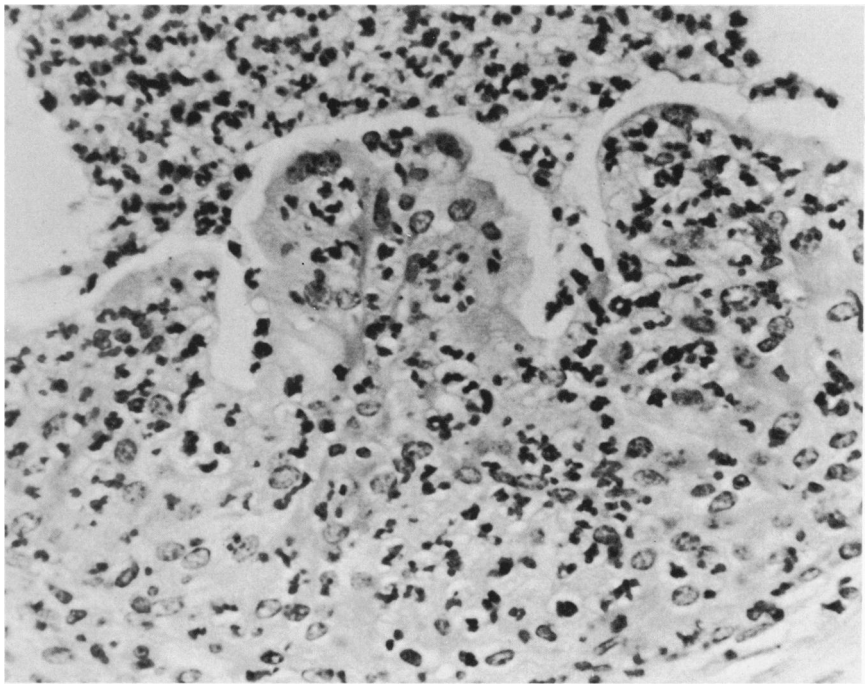


Fig 7. Photomicrograph of lesion involving duct of cauda epididymis showing heavy infiltration of PMNs. Testis taken from guinea pig 15 days after immunization with ASPM in CFA. Periodic acid-Schiff hematoxylin stain. × 500.

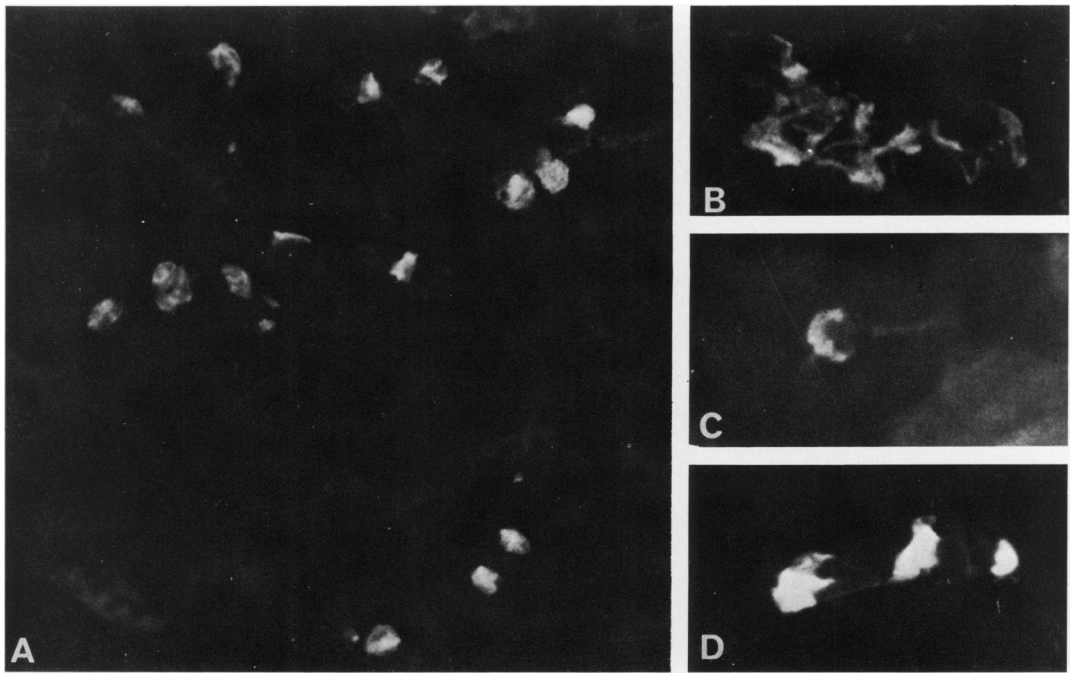


Fig 8. Immunofluorescence photomicrograph of rete testes of 4 guinea pigs immunized with ASPM stained with fluorescein-labeled sheep anti-guinea pig IgG. Note brightly stained acrosomes that appear distorted or clumped together. These guinea pigs were studied at (**A**) 12, (**B**) 15, (**C**) 16 and (**D**) 18 days after immunization with ASPM in CFA. **A,** × 400; **B, C** and **D,** × 600.