Localization of Achrosomal Antigenicity in Guinea Pig Testes.* (27816)

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Morphologic damage of the adult guinea pig testis can be induced by the single intracutaneous injection of homologous testes extract plus complete Freund adjuvants(1-3). The characteristic feature of this phenomenon is sloughing of the germinal epithelium so extensive as to result in complete aspermatogenesis. Vacuolization of spermatogonia, pycnosis of germ cell nuclei, appearance of multinucleated cells and cytoplasmic vacuolization of Sertoli cells have been related morphologic findings; no influence on the Leydig cells has been observed. That this reaction is due to an immunologic mechanism has been amply demonstrated. Various authors have reported the presence of circulating antibodies (1), delayed cutaneous hypersensitivity(1) and effectiveness of passive transfer by white cells from sensitized donors(4). Attempts have been made to characterize chemically the antigen responsible for the induced aspermatogenesis. Since the initial work of Freund(1) attention has been focused upon the carbohydrate rich achrosome and it has been known that polysaccharide fractions of testicular extracts are active. Other sperm antigens have been identified including hyaluronidase, a nucleic acid containing substance and at least one unidentified protein. According to Katsh, both the polysaccharide and the nucleic acid antigen are capable of inducing aspermatogenesis(5). It should be mentioned, however, that no claims of absolute purity have been made for these various fractions. Although attempts have been made to localize the cellular depots of active antigen this problem requires clarification and this work deals with this aspect of immunologically induced aspermatogenesis.

Material and methods. Testes removed from normal adult guinea pigs were homogenized with volumes of saline equal to the weight of the testes and the homogenate emulsified in an equal amount of complete Freund adjuvant. The experimental group consisted of 26 adult male guinea pigs (lot No. 1). Each recipient animal was injected intradermally, in the neck region, with 1.0 ml of the mixture administered in 10 sites (0.1 ml per site). An additional 6 animals received 1.0 ml of testis extract without adjuvant (lot No. 2) and 6 were injected with 1.0 ml of adjuvant without testis extract (lot No. 3). The animals were exsanguinated from the heart between 40 and 60 days after the injection. Individual sera were tested for antibody titer by complement-fixation using homologous testicular saline homogenate. Those sera showing positive complement-fixation results were pooled and used in the experiments. Separate pools were made of sera from animals in the 2 control groups. A portion of each serum pool was fractionated by the cold-ethanol method and the globulin fractions were collected. Whole normal guinea pig serum was fractionated by cold-ethanol precipitation and separated albumin, globulin and fibrinogen were collected. These fractions were precipitated with alum-chloride and used to immunize adult male rabbits.

Whole guinea pig sera or the globulin fractions, as well as the rabbit antisera, were labelled with fluorescein isothiocyanate using the method described by Riggs(6). The conjugates were absorbed 2 times with an acetone dried powder of homologous liver using 100 mg powder per 1.0 ml of conjugated antiserum.

Fresh frozen testicular sections of sensitized as well as normal guinea pigs were used to perform both the direct (1-step) and indirect (2-step) Coons technic(7). The direct procedure involved incubation of normal testicular sections with: a) labelled immunoglobulins from pooled sera of lot No. 1; b) labelled normal globulins; c) unlabelled im-

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munoglobulins from pooled sera of lot No. 1 followed by labelled immunoglobulins (blocking action); d) rabbit antiguinea pig albumin; e) rabbit antiguinea pig globulin; f) rabbit antiguinea pig fibrinogen (Table I). In another experiment testicular sections of

TABLE I. Fluorescent Antibody Localization in Normal Guinea Pig Testes.

	FIGG	FNG	UIGG + FIGG	FAGA	FAGG	FAGF	FIGGA
Albuguinea	—	—	—	±	+	—	—
Blood vessels	—	—	—	±	+	—	—
Achrosome of spermatids	+++	±	±	±	±	—	±
Head of spermatozoa	+++	±	±	±	±	—	±

Key: FIGG, fluorescent immuno guinea pig globulin; FNG, fluorescent normal guinea pig globulin; UIGG, unlabelled immuno guinea pig globulin; FAGA, fluorescent antiguinea pig albumin (rabbit); FAGG, fluorescent antiguinea pig globulin (rabbit); FAGF, fluorescent antiguinea pig fibrinogen (rabbit); FIGGA, fluorescent immunoglobulins absorbed with testis powder.

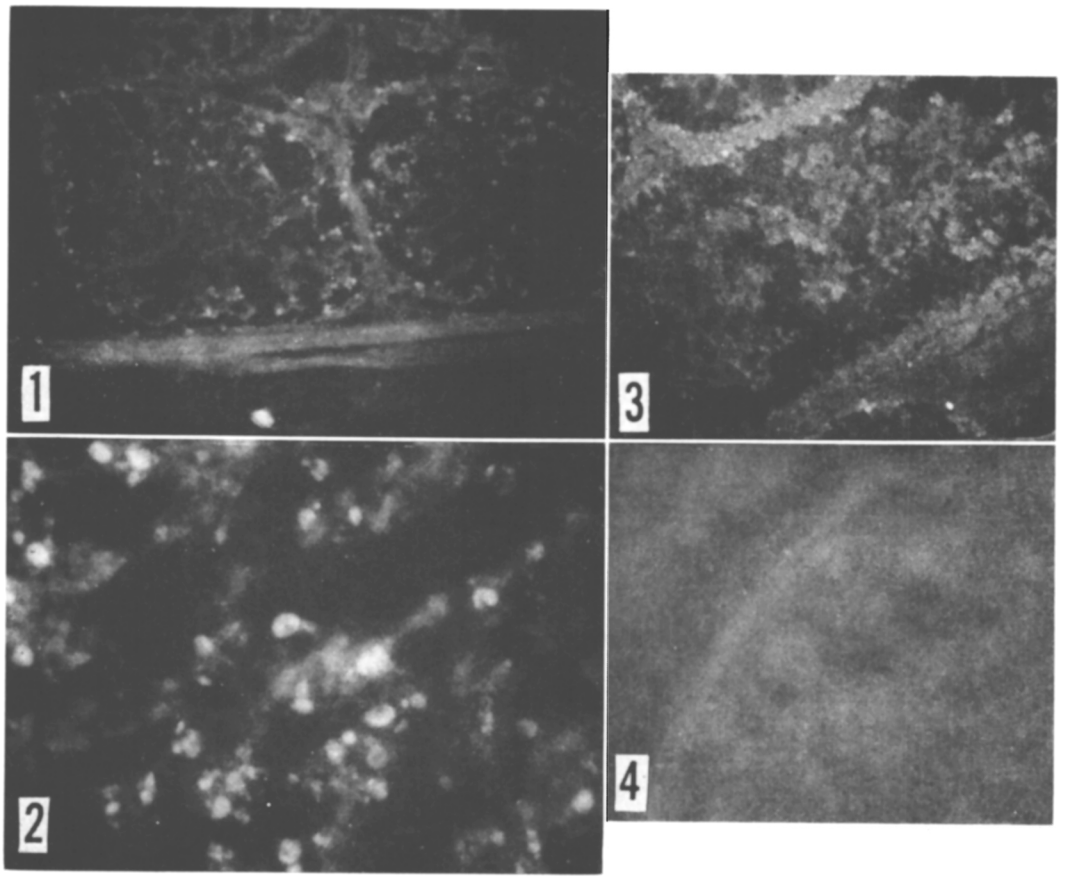

FIG. 1. Fresh frozen testicular section of a guinea pig from Lot #1 incubated with his own serum followed by fluorescent antiguinea pig globulin. Specific fluorescence can be seen in germinal cells. The tubular wall shows no stain.

FIG. 2. Higher magnification of same section as Fig. 1. Specific fluorescence can be detected as bright spots of different shape corresponding to different stages of achrosome development.

FIG. 3. Fresh frozen testicular section of a guinea pig from Lot #1 incubated with normal serum followed by fluorescent antiguinea pig globulin. Only weak staining correspondent to autofluorescence can be observed.

FIG. 4. Higher magnification of Fig. 3. No specific distribution of fluorescence can be detected.

TABLE II. Fluorescent Antibody Localization in Experimental Guinea Pig Testes.

Exp groups	Structure of testis	Fluorescent antibody technics: Direct FAG	FNG	FAGA	FAGG	FAGF	Indirect UAS + FAGA	UAS + FAGG	UAS + FAGF	UNS + FAGA	UNS + FAGG	UNS + FAGF
Lot #1	Albuginea	−	−	++	++	−	−	−	−	−	−	−
	Blood vessels	−	−	++	++	−	−	−	−	−	−	−
	Ach. spermatids	++	++	++	++	−	++	++	−	++	++	−
	Head sperm	++	++	++	++	−	++	++	−	++	++	−
Lot #2	Albuginea	−	−	++	++	−	−	−	−	−	−	−
	Blood vessels	−	−	++	++	−	−	−	−	−	−	−
	Ach. spermatids	++	++	++	++	−	++	++	−	++	++	−
	Head sperm	++	++	++	++	−	++	++	−	++	++	−
Lot #3	Albuginea	−	−	++	++	−	−	−	−	−	−	−
	Blood vessels	−	−	++	++	−	−	−	−	−	−	−
	Ach. spermatids	++	++	++	++	−	++	++	−	++	++	−
	Head sperm	++	++	++	++	−	++	++	−	++	++	−

Key: FAG, fluorescent autoglobulins; UAS, unlabelled autologous sera; UNS, unlabelled normal sera. The key for the remaining abbreviations may be found on Table I.

animals of each group were incubated with: a) autologous or homologous unlabelled sera followed by rabbit fluorescent antiguinea pig globulin; b) the labelled globulin fraction of the above mentioned sera; c) rabbit anti-guinea pig albumin, globulin or fibrinogen.

Results. In the sera of 9 animals of lot No. 1 complement-fixing antibodies ranging in titer from 1:8 to 1:32 could be demonstrated. None of the sera from animals of lots No. 2 and No. 3 had antibodies. Among the 26 animals of lot No. 1 20 showed mild to severe damage of the germinal epithelium. No testicular damage was evident among the controls. Table I shows location of specific fluorescence in normal testicular sections incubated with pooled labelled globulins from animals of lot No. 1. The head of spermatozoa as well as the achrosome of spermatids appear brightly stained (Fig. 1, 2). Similar results were found when the whole labelled serum was used instead of its globulin fraction. The same structures in sections subjected to blocking with immune unlabelled globulins or incubated with normal labelled globulins show only a weak fluorescence (Fig. 3, 4). Table II shows location of fluorescence when testicular sections of each group were incubated with homologous sera or globulin fraction using both direct and indirect methods. It should be emphasized that in some damaged tubules belonging to testes of sensitized animals, yellow-greenish fluorescent masses could be seen in the lumen. This should not be confused with specific fluorescence. Identification of the histologic structure associated with fluorescence can be confirmed by comparison of adjacent stained sections. It may be noted that the spermatid achrosome and the sperm head are the only cytological units that invariably showed predominant fluorescence, regardless of the technic involved in the localization of antibodies. The fluorescence in this area is always considerably greater with immune globulins than the low intensity of fluorescence sometimes associated with normal globulins or antibodies against guinea pig serum fractions. Furthermore, the blockage effect by pretreatment with unlabelled antibodies (before addition of fluorescent antibodies) is more pronounced with respect to achrosome and sperm head (Table I). In no instance is significant fluorescence associated with albuguinea and blood vessels. When the immune serum is absorbed *in vitro* with lyophylized testes powder and then used in Coons technic, the specific fluorescence does not appear. Similar extracts of guinea pig kidney and

liver are not able to remove the specific antibodies from the serum.

Discussion. Homologous testes antisera contain antibodies that localize with the achrosome of the spermatid and the head of spermatozoa. This specific localization occurs in the testes of sensitized as well as unsensitized animals. It would appear, therefore, that the autoantigen constituent of testes extracts resides in this cytological component. This finding confirms by fluorescent technics the suggestion of Freund *et al.*(1) and Katsh(5) that aspermatogenic antigen may be a polysaccharide associated with the achrosome. The results are in agreement also with those of Baum(8) whose pictures indicate localization of fluorescence in the mature sperm but do not permit identification of specific cytological regions. The low grade fluorescence we observe in the albuguinea and blood vessel walls can be expected on the basis of previous experiments. It has been shown that normal serum proteins deposit to a limited extent in the extra-vascular connective tissue structures(9). Further evidence for the specificity of fluorescent localization in the spermatid achrosomes and sperm head is provided by the absorption experiments in which lyophylized guinea pig testes are effective in removing antibodies while liver and kidney extracts are not. The lack of correlation between circulatory levels of antibodies and morphologic damage to the testes is similar to the results reported by other investigators and points to the fact that much remains to be elucidated with respect to the mechanisma of the immunological insult to the testes as well as the chemical identification of antigens involved in the induction of aspermatogenesis.

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Influence of Host Age and DNA Precursors on Intracellular Staphylococci.* (27817)

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Previous investigations on the intracellular fate of virulent staphylococci in monocytes from "normal" adult rabbits(1,2,3) had shown that virulent *S. aureus* organisms survive intracellularly for some time but are eventually destroyed. No intracellular multiplication was observed in these studies, employing monocytes maintained *in vitro,* nor in the studies of others (see 3). If one assumes that staphylococci have a basic potential for intracellular residence and multiplication, then the observations with phagocytes from adult rabbits suggest the possibility that failure of multiplication in cells from the adult host may be due to the host's natural attainment of a considerable degree of resistance as a result of prior natural exposure. If this were true then monocytes from newborn rabbits, which presumably lack signifi-

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