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Passive transfer of autoimmune aspermogenic orchiepididymitis (AIAO) by antispermatozoa sera

INFLUENCE OF THE TYPE OF AUTOANTIGEN AND OF THE CLASS OF ANTIBODY

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SUMMARY

Three different autoantigens (S, P and T), extracted and separated from guinea-pig spermatozoa, give rise to an autoimmune aspermogenic orchitis (AIAO) when injected with Freund's complete adjuvant (FCA). They also induce specific antibodies, such as anaphylactic (with S and P), complement-fixing (with P and T), spermotoxic (only with T) and precipitating and Arthus-inducing antibodies (only with P).

Passive transfer of AIAO was attempted by injections of high total doses (15–20 ml per animal) of immune sera directed against one of the three antigens. Successful passive transfers were evaluated by the intensity of the epididymal and testicular lesions which were comparable to the actively induced ones, and by the rapid appearance of these lesions in less than 1 week and their lasting for at least 2 weeks. The disease was passively transferred with anti-P immune sera in as many as 64% of these cases and up to 40% with anti-T immune sera. Anti-S sera did not transfer AIAO more than did control normal and anti-DNP-BGG guinea-pig sera.

The incidence and intensity of lesions were greatly for anti-P or slightly for anti-T increased by pretreating the future recipients with FCA.

Hyperimmune sera are considerably more effective than early sera even when the latter are used in a time sequence reproducing that of the active reaction.

The orchitogenic activity of anti-T sera appears to be localized in IgG2 DEAE fractions while that of anti-P has been found only in IgG1-containing DEAE fractions.

INTRODUCTION

The nature of the immune agents responsible for auto-immune lesions, especially AIAO, is still a controversial subject. Transfer experiments have been successfully performed with lymphoid cells (Lerner, Stone & Goode, 1969; Tung, Unanue & Dixon, 1971a), or immune sera (Pokorna, 1969 and 1970; Tung, Unanue & Dixon, 1971b; Willson, Jones & Katsh, 1972; Nagano & Okumura, 1973), or certain combinations of both, such as specific antibodies in animals with delayed hypersensitivity or immune cells into hyperimmune animals (Brown, Glynn & Holborow 1967; Toullet & Voisin 1969). Different immunological mechanisms may play diverse roles in varying situations, especially with dissimilar antigens; therefore, separation of these antigens is a prerequisite to study this question.

At least four autoantigens (S, P, T and Z) exist in one single type of cell, namely guinea-pig spermatozoa. Three of these antigens (S, P and T) have been separated and shown to induce lesions of AIAO when injected with complete adjuvants (Voisin & Toullet, 1968). These three autoantigens have different physico-chemical and immunological properties (Voisin & Toullet, 1968; Toullet & Voisin, 1974;

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Toullet, Voisin & Nemirovsky, 1973). The lesions of AIAO induced by them are indistinguishable from each other when they are at their mature stage; however, they differ at the beginning, according to the immunising antigen, with aspects suggesting that the initial immune mechanisms may be different (Toullet; Voisin & Nemirovsky, 1970), involving mainly either cells or antibodies.

The preceding evidence lead to the present experiments where passive induction of AIAO lesions was attempted by means of anti-S, anti-P and anti-T homolous immune sera injected into adult male guinea-pigs.

A striking difference was found between the three auto-antigens as regards the capacity of the corresponding antibodies, as well as their immunoglobulin classes, to transfer orchiepididymal lesions. Successful transfers were facilitated by pretreating the recipients with FCA.

MATERIALS AND METHODS

Animals. Adult male Hartley guinea-pigs (400–500 g) were used as donors and recipients of immune serum.

Immunizing autoantigens. S, P and T, were prepared as previously described (Voisin & Toullet, 1968; Toullet & Voisin, 1974). Briefly, soluble S and P autoantigens were extracted from guinea-pig spermatozoa ground in distilled water, and separated by 5% trichloracetic acid (TCA) into one soluble fraction, S (in supernatant) and one insoluble fraction, P (in precipitate). TCA-soluble S was purified on Sephadex G-100 from which it is excluded. After dissolution in diluted NaOH, the proteins of the TCA insoluble fraction were precipitated by a chloroform–butanol mixture; autoantigen P remained in the aqueous phase and was purified by filtration on Sephadex G-100 in which it is retained. Hydro-insoluble T autoantigen was separated from spermatozoa and debris by differential centrifugation, sonicated and repeatedly washed to eliminate soluble antigens.

Autoantibodies anti-S, P and T autoantigens. These were prepared as follows: castrated male guinea-pigs were given a first injection of autoantigens (dose equivalent to 108 spermatozoa) with Difco FCA in the hind footpads and nuchal region. At monthly intervals they received three more injections s.c. without adjuvant. Serum was taken either 10–15 days after the first injection ('early antibodies') or 5–7 days after the last one ('late or hyperimmunization antibodies'). Pools were made of early or late antibodies after antibody titration of each antiserum. Antibody activity titres were determined by passive haemagglutination for anti-S, and anti-P, and by sperm immobilization and cytotoxicity (Toullet & Voisin 1974), for anti-T. Titres and properties are shown in Table 1. Each pool is inactive against the two other autoantigens. In half of the cases, the pools were concentrated by 50% ammonium-sulphate precipitation, increasing their titres by a factor of 4.

Control sera included sera from normal untreated male guinea-pigs, antidinitrophenyl-bovine gammaglobulin (DNP-BGG) guinea-pig sera (complement fixation titre = 4096) and anti-ovalbumin (OVA) guinea-pig sera (haemagglutinating titre, 6×10^4; haemolytic titre, 2560). Concentrated globulins were also used.

TABLE 1. Titre and properties of the pools of immune sera directed against, S, P or T autoantigens utilized for passive transfer of AIAO

Specificity of antibodies	Nature of test	Titre of pools		Other properties of antibodies
		Early antibodies	Late antibodies	
Anti-S	Passive haemagglutination	128*	1000	Anaphylactic Non-precipitating Non-C1 fixing Non-spermagglutinating
Anti-P	Passive haemagglutination	64	100,000	Anaphylactic Complement fixing Precipitating (only late antibodies) Non-spermotoxic Non-spermagglutinating
Anti-T	Spermotoxicity	100	400	Spermagglutinating Complement fixing Cytotoxic for testicular germinal cells

* Titre (reciprocal of dilution).

Fractionation of anti-P and anti-T immune sera. This was done on DEAE cellulose columns in order to separate IgG1 and IgG2 antibodies. Briefly, 40–45 ml of serum, dialysed against buffer, were applied on a 500-ml column of DEAE cellulose equilibrated with 0·005 M pH 7·5 phosphate buffer. After effluent collection (IgG2) and washing, a NaCl-molarity gradient up to 0·25 M was applied and 20-ml fractions collected. All fractions were examined for optical density at 280 nm and conductivity. After having been rendered isotonic, the presence of different types of anti-P or anti-T antibodies were tested by the following methods: passive haemagglutination and haemolysis, as well as PCA, for anti-P and spermoagglutination and spermotoxicity, as well as complement-fixation for anti-T. According to their molarity and immunobiological properties, the fractions were pooled into five or six pools. Each one was tested again for antibody properties and titration as well as for the presence of immunoglobulins of different classes (Ouchterlony with monospecific anti-IgG1, IgG2 and IgM immune sera).

One of these pools containing IgG and IgM (anti-P, pool IV) was filtrated on Sephadex G-200 in order to separate IgM from IgG.

Separation of 7 S and 19 S globulins of an anti-P hyperimmune serum was done in a 10–40% sucrose gradient after 18-hr centrifugation in a SW 39 rotor at 35000 rev/min.

Experimental design. Either whole serum or DEAE chromatography fractions were used for passive transfer.

1. *Whole serum experiments.* On day −14, part of the prospective serum recipients received 0·5 ml of FCA in hind footpads and nuchal region, prepared as follows: Freund's incomplete adjuvant (0·25 ml), killed tuberculi bacilli (human 0·5 mg+bovine 0·5 mg), saline (0·25 ml) and 5 μg of ovalbumine (OVA). The latter was omitted sometimes.

On day 0 and +2, an i.v. injection of 4–6 ml of serum or concentrated globulins was given to the recipients together with 2·5–5 ml of same preparations s.c. Each recipient received a total of 15–20 ml of serum or globulins.

On day +6, 7, 9, 10 or 13, the recipients were given 0·24 ml/100 g of weight of a 5‰ solution of Evans blue to test the state of epididymal and testicular vascular permeability. Two hours later, sera were collected for serological study (titre of injected antibodies), the animals were killed and the testis and epididymis were removed and fixed in acetic Bouin for histology. One day and also 10 min before sacrifice, the animals received one injection of S and one of P (dose equivalent to 10^7 spermatozoa in 0·1 ml), as well as one of OVA (10 μg) to study cutaneous hypersensitivity reaction of the delayed, Arthus and anaphylactic types according to an already described convenient and reliable method (Voisin & Toullet 1966).

In one experiment, one testis was examined 24 hr after the first serum injection and the other one 5 days later (3 days after the second serum injection).

2. *DEAE fractions experiments.* All recipients were pretreated with FCA. All were injected i.v. during 3 consecutive days. Each pool was administered into the vein of one single recipient. Controls received either normal or anti-ovalbumin serum (dialysed against 0·005 M, pH 7 phosphate buffer, re-equilibrated with ClNa for isotonicity) or ammonium-sulphate-precipitated globulins. Hypersensitivity tests and histological examination were similar to those applied to whole serum recipients.

RESULTS

Description of passively induced lesions

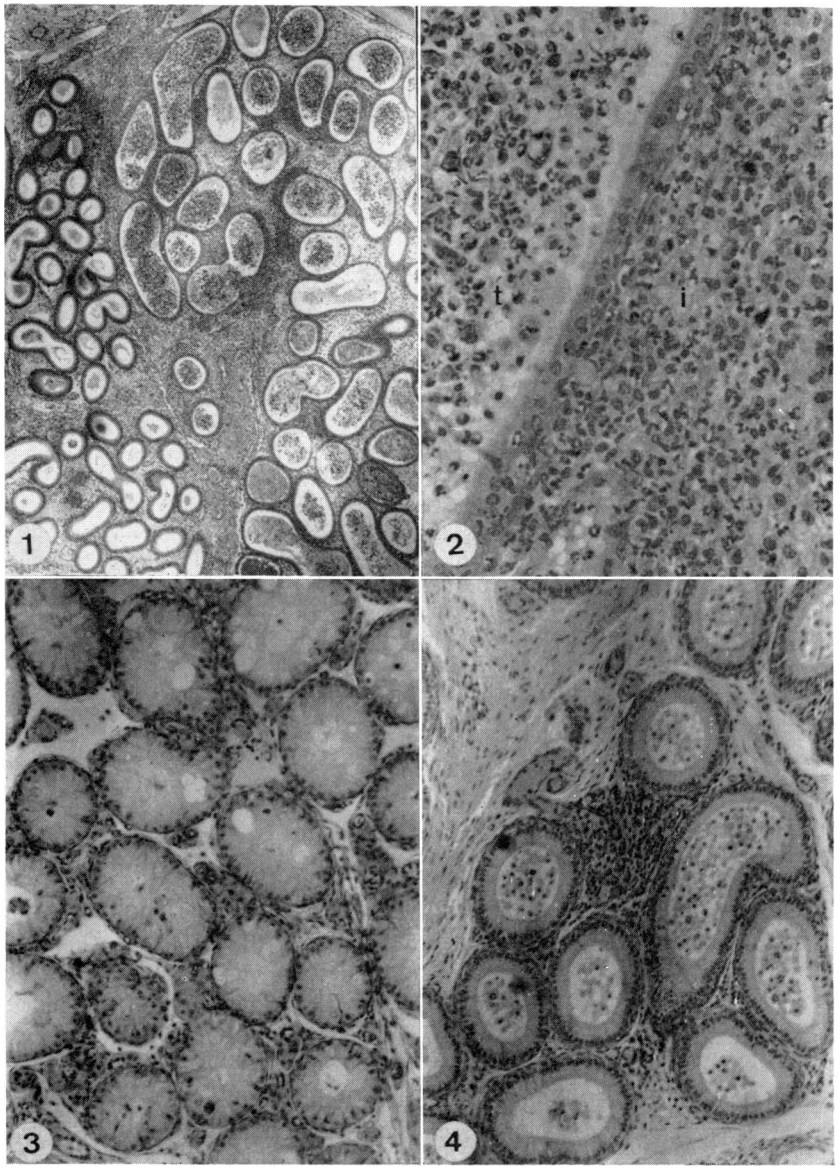
Two types of lesions, cellular infiltrates in the epididymis and aspermatogenesis in the testis, were observed, often found together, as in actively induced lesions.

Epididymal infiltrates. These consisted of mononuclear cells or of polymorphonuclear leucocytes (PMNs) or both. Slight to moderate, even dense, infiltrates, mainly made of mononuclear cells, and restricted to perivascular areas were seen occasionally. In other cases, important infiltrations were detected, with numerous cells, mainly PMN, passing between the epithelial cells and invading the lumen of the epididymal tubules (Figs 1, 2, 5, 9 and 11). These infiltrates were sometimes so conspicuous as to look like abscesses (Fig. 5), often also found in active immunization, especially with *P. oedema* was also present (Fig. 5) and increased vascular permeability was detected by Evans blue extravasation, in all cases with cell infiltrates.

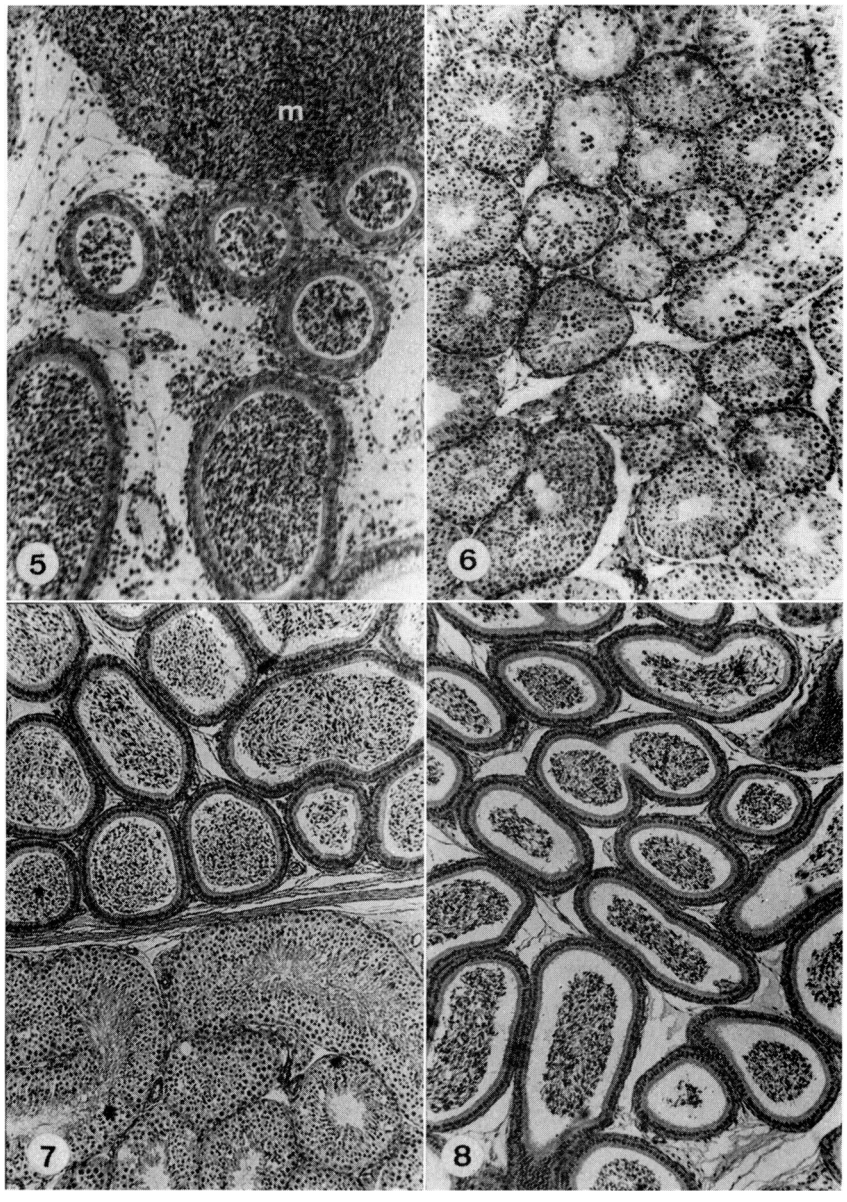
Testicular aspermatogenesis. This was moderate, without cell infiltrates in the interstitium (Figs 6, 12) and almost no alterations in the rete testis, with the exception of two cases treated with anti-P preparations (one pool of immune sera and one DEAE fraction) (Figs 3 and 10).

The overall intensity of lesions has been classified as follows: mild lesions without Evans blue, with small cellular infiltrates and slight aspermatogenesis; medium lesions with moderate or strong cellular infiltrates, moderate aspermatogenesis usually present; strong lesions, Evans blue present, strong cellular infiltration, subtotal or total aspermatogenesis.

As a whole, twenty-six (28·9%) of the ninety animals having received various preparations of anti-spermatozoa autoantigen immune sera or globulin fractions presented lesions, while only three out of the fifty-four (5·6%) receiving control preparations did so.



FIGS 1–4. Epididymitis and aspermatogenesis in guinea-pigs pretreated with FCA and injected i.v. 2 weeks later with anti-P serum. FIG. 1: Six days after injection of antibody, oedema and infiltration of epididymis with many polymorphonuclear and some mononuclear cells. (Magnification × 11.) FIG. 2: On the same date, a higher magnification showing intersititium (i) invaded by polymorphonuclear and mononuclear cells and the lumen of a tube (t) filled with degenerated spermatozoa had neutrophils. (Magnification × 112.) FIG. 3: Six days after injection of antibody, complete aspermatogenesis of testis. (Magnification × 36.) FIG. 4: Thirteen days after injection of antibody, the fibrosis has replaced oedema in the epididymis and only mononuclear cells are present: exfoliated germinal cells fill the epididymal tubules (Magnification × 28.)



FIGS 5–8. Epididymitis and aspermatogenesis in guinea-pigs pretreated by FCA and injected i.v. 2 weeks later with immune sera or globulins directed against various spermatozoal autoantigens. FIG. 5: Six days after injection of anti-T serum, oedema of epididymis, invasion of the tubes by PMN, huge mass (m) of degenerated tubes and PMN (Magnification × 45.) FIG. 6: Six days after i.v. injection of anti-T serum, moderate aspermatogenesis in the testis. (Magnification × 28.) FIG. 7: Six days after i.v. injection of anti-S serum: no lesion of epididymis and testis. (Magnification × 28.) FIG. 8: Seven days after i.v. injection of anti-P IgG2, no lesion is observed in the epididymis. (Magnification × 28.)

TABLE 2. Incidence of orchi-epididymitis induced by antispermatozoa autoantigens S, P, T, sera in guinea-pigs

Type of serum used	Immune sera				
	Anti-P serum	Anti-T serum	Anti-S serum	Anti-DNP-BGG serum	Normal serum
Early sera	2/10 (20)	3/9 (33)	0/8 (0)		
Late sera	10/25 (40)	2/8 (25)	2/14 (15)	1/17 (6)	
Total	12/35 (34)	5/17 (29)	2/22 (9)	1/17 (6)	2/27 (7·4)

The donor guinea-pigs were submitted to either a 2-week immunization with a single dose of antigen (early sera) or a 3–4-month immunization with repeated injections (late sera).
Figures in parentheses are percentages.

Other organs. Those examined (kidney and thyroid) presented no lesions with the exception of three animals injected with FCA (two controls that had received anti-DNP-BGG and one experimental that had received anti-P). These three animals presented one small mononuclear infiltrate in either the thyroid or the kidney or both. None had testicular or epididymal lesions.

Immunological specificity of responsible antibodies. Influence of the type of antigen

Incidence of lesions in the recipients of immune sera (Table 2). Lesions of epididymitis followed the injection of anti-P and anti-T sera (34% and 29%, respectively) showing essentially mononuclear cells, sometimes associated with a large number of PMN (Figs 1, 2, 4, 5, 9 and 11). Anti-autoantigen S sera rarely produced the same lesions; when present, they consisted of mononuclear cells located in the epididymis. This effect was not significantly different from that of normal or anti-DNP-BGG sera (Fig. 7).

The incidence of the lesions, vascular permeability increase and cellular invasion of the epididymis suggest that long term immunization appears to be more efficient. The highest incidence of epididymitis occurred with anti-P late serum (40%) as compared to early serum (20%).

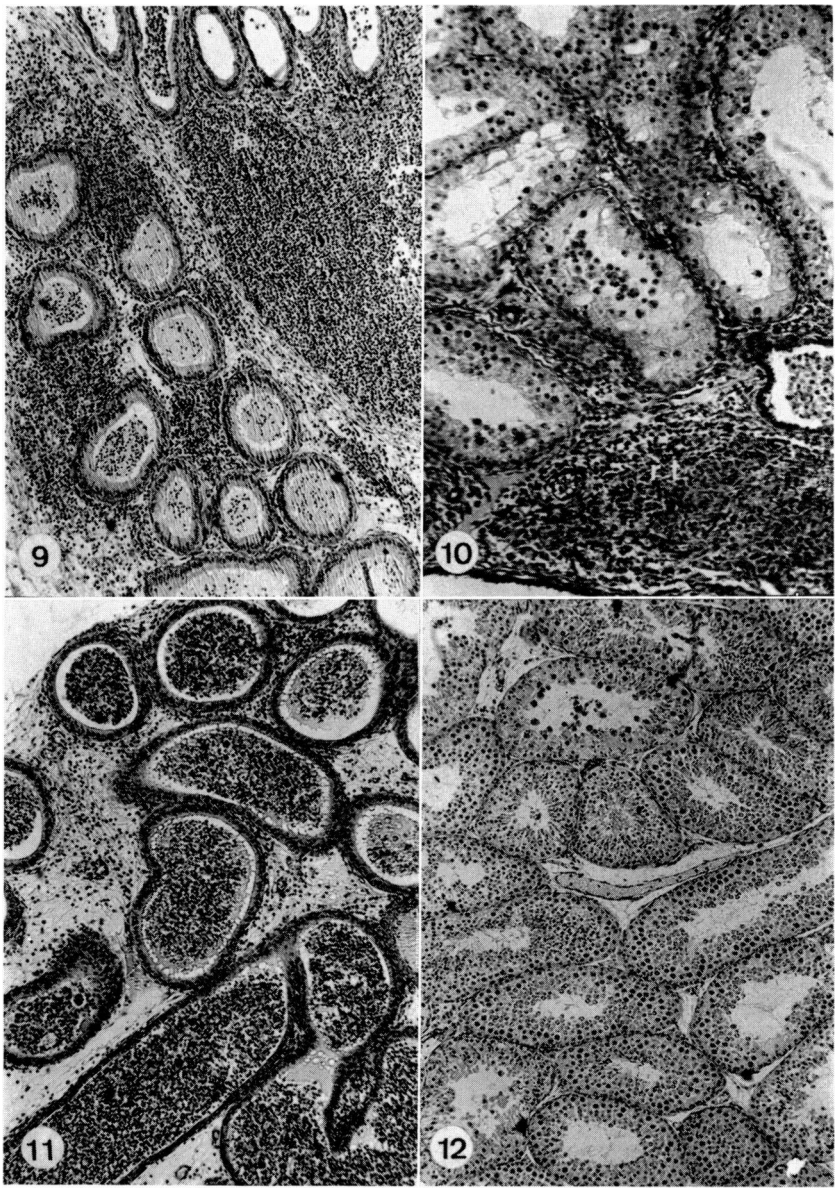
Passive immunological reactivity of the serum recipients

Cutaneous hypersensitivity. Strong anaphylactic reactions were elicited by S and P antigens in the recipients of early and late anti-S and anti-P sera. Recipients of late anti-P sera showed Arthus reactions, while atypical, non-haemorragic, 'white Arthus' (Maillard & Voisin, 1970) was observed in recipients of early anti-P, as well as early—and late—anti-S sera (Table 3). As expected, no delayed hypersensitivity was found. Because of the immediate inflammatory reaction produced in the skin of normal animals by

TABLE 3. Cutaneous hypersensitivity reactions in recipients of anti-autoantigens S and P

Type of immune serum injected		Number of injected animals	Number of animals with the following reactions:		
			Anaphylaxis	Haemorrhagic Arthus reaction	Non-haemorrhagic Arthus reaction ('White Arthus')
Anti-P	Late serum*	21	21	17	4
	Early serum	7	7	0	4
Anti-S	Late serum	10	10	0	10
	Early serum	6	6	0	2

* For definition of the terms *late* and *early*, see preceding table.



FIGS 9–12. Histological changes of epididymis and testes of guinea-pigs pretreated with FCA 2 weeks before i.v. injection of immunoglobulins derived from DEAE cellulose chromatography fractions of immune sera. FIG. 9: Seven days after i.v. injection of anti-P IgG1 fractions (pool IV). Epididymis is invaded by polymorphononuclear and mononuclear cells sometimes organized as an abscess (a) after destruction of tubules. (Magnification × 28.) FIG. 10: Section of rete testis (rt) and testis of the same guinea-pig as in Fig. 9. Invasion of rete testis by polymorphonuclear and mononuclear cells, strong exfoliation of germinal cells. (Magnification × 45.) FIG. 11: Seven days after i.v. injection of anti-T IgG2 fractions (pool I), oedema, polymorphonuclear and mononuclear cells are present in epididymis outside and inside the tubules. (Magnification × 28.) FIG. 12: Exfoliation of germinal cells in the testis of the same guinea-pig as in Fig. 11. (Magnification × 28.)

autoantigen T, the cutaneous hypersensitivity to this antigen was not studied in the recipients of immune sera.

Serum antibodies. The transferred antibodies were readily detected in recipients of late sera. Haemagglutination titres reached 5000–40,000 for anti-P antibodies and 32–256 for anti-S sera; anti-T antibodies gave titres of 10–100 in spermotoxicity tests. The already weak early sera produced only threshold titres in the recipients.

Influence of pretreating the prospective recipient with FCA

This pretreatment induced, as a whole, an increase in the percentage of observed lesions (Fig. 13). More precisely, the pretreatment of animals receiving hyperimmune anti-P sera increased the incidence of lesions from 9–64% ($P<0{\cdot}01$) and the intensity of the epididymal lesions, with augmented vascular permeability and PMN invasion (Figs 1, 2). In forty-four controls receiving normal or anti-DNP-BGG sera the only three cases presenting some degree of lesions had previously received injections of FCA. Another three recipients presenting small mononuclear infiltrates only in kidneys and/or thyroid were

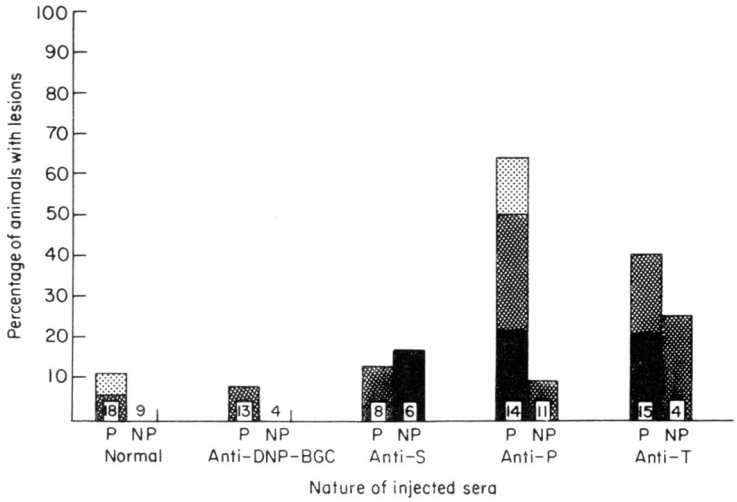


FIG. 13. Passive transfer of AIAO by hyperimmune sera directed to spermatozoa autoantigens. Influence of pretreating the prospective recipients with FCA on the percentage of observed lesions. P, Pretreated (FCA) recipients; NP non-pretreated recipients. Numbers in squares are the number of recipients. Columns = degree of lesions, stippled = mild, blue −; infiltration and aspermatogenesis ±; cross-hatched = medium, blue + or −, infiltration + or ++; solid = strong, blue +, infiltration ++, aspermatogenesis.

also pretreated with FCA. However, FCA did not appear to influence lesions in guinea-pigs receiving either sera of early immunization (anti-S, P or T) or hyperimmune anti-S serum. An analogous, although less marked, increase in recipients of hyperimmune anti-T sera seemed to exist but seemed to be of no significance. Changing the time interval from 14–7 days between FCA pretreatment of recipients and serum injections did not increase significantly the incidence of positive transfers.

As a variation to the above described experiments the following protocol was designed: sixteen guinea-pigs were actively immunized with 1-month course of injections of autoantigen P or T, alone or with Freund's incomplete adjuvant,—a procedure known to give rise to some antibody production, although not as much as with FCA and of restricted type—without delayed hypersensitivity and with no lesions of AIAO. FCA were administered to one half of the animals 1 week after the end of the immunization in order to see whether this procedure would induce the disease in these animals possessing circulating antibodies already formed in the absence of FCA. In this model, no disease was observed in the organs examined 10 or 17 days after FCA with one exception: one of the four guinea-pigs treated with antigen T and later with FCA showed some alteration consisting of eosinophil leucocytes and mononuclear cells extravasated in rete testis and epididymis. The antibody titres achieved by this experimental procedure remained very low.

TABLE 4. Influence of interval between serum injection and examination in the recipients on the incidence of AIAO lesions

Specificity of injected sera	Pretreatment with FCA	Interval between the first injection of sera and the examination of epididymis and testis								Total	
		Day 1		Day 6 or 7		Day 9 or 10		Day 13			
Anti-P	+	1/2		6/11		1/3		2/7		10/23	
	−		1/2		0/10		0/2		1/1		2/15
Anti-S	+	0/2		0/5		1/8				1/15	
	−		0/2		0/5		1/4				1/11
Anti-T	+	0/2		1/3		2/7				3/12	
	−		0/2		1/3		1/4				2/9
Anti-DNP-BGG or normal serum	+	1/2		1/12		0/12		1/6		3/32	
	−		0/1		0/8		0/3		0/2		0/12

Data concerning late and early immune sera have been added in the table.

Relation between time of serum injection and examination of the recipients (Table 4)

Twenty-four hours after the injection of immune sera, no modification was found in the organs except when anti-P sera had been administered, where few mononuclear cells and eosinophils had extravasated in the rete testis region. Clear epididymal and testicular lesions were seen on the 6th day. Their incidence, intensity and aspect did not seem to vary from 6–13 days after initiation of serum treatment. However, in animals treated with FCA plus anti-P hyperimmune serum, PMN present in the epididymis from day 6 to day 10 had disappeared on day 13 (Fig. 4).

Immunoglobulin class of the responsible antibodies

Hyperimmune 'late' sera were submitted to preparative DEAE cellulose chromatography fractionation aimed at defining the pathogenic capacity of the two main IgG classes, IgG1 and IgG2.

Fractionation of anti-P sera (*Table 5*). Of the five pools obtained from each of three different columns, each loaded with 45 ml of serum, pool I contained IgG2 antigenically pure (Ouchterlony test with anti-IgG1, IgG2 and IgM); pools IV and V contained IgM but pools II to V carried a mixture of IgG1 and IgG2. From the biological point of view, a complement-fixing property with antigen P was found in pools I and II and anaphylactic properties in pools II to V.

Among all the materials described above only one, pool IV, produced a manifest disease consisting of an obvious vascular permeability increase and of mononuclear and PMN cells invading the epididymis even organized in pseudoabscesses (Fig. 9). In one of the two animals studied, the mononuclear infiltrate extended to the testis which appeared devoid of germinal cells (Fig. 10). Fractions other than IV could not produce noticeable lesions (Fig. 8) nor did the mixture of half doses of I (complement-fixing IgG2 antibodies alone) and III or IV (containing IgG1 anaphylactic antibodies).

No lesions were produced by any pool from another fractionation starting with only 25 ml of anti-P sera, a lack of effect probably due to subpathogenic amounts of antibody. Finally, in order to test the role of IgM, a Sephadex G-200 filtration was made of fraction IV (able to produce the disease) but no anti-P activity was found in the excluded globulins. Furthermore, the 19 S fractions of a sucrose gradient separation made of an anti-P hyperimmune serum was similarly devoid of anti-P activity. All the activity was present in the 7 S fractions.

The efficiency of the antibody transfer was attested by the anaphylactic reactions obtained in the recipients of pools II to V and the Arthus reactions in recipients of pools III to V. There was no correlation between the intensity of the Arthus reaction and the lesions, especially since the non-pathogenic mixture of pools I+III or I+IV gave rise to larger Arthus reactions than pathogenic pool IV.

TABLE 5. Pathogenic capacity of fractions obtained by DEAE-cellulose chromatography of anti-autoantigen P sera

Pools	Presence† of			Injected volume (ml)		Total haemagglutination units¶ ($\times 10^{-4}$)		Total haemolytic units ($\times 10^{-4}$)		Total anaphylactic units ($\times 10^{-4}$)		Lesions of epididymis and testis in recipients	
	IgG2	IgG1	IgM	1st expt	2nd expt	1st expt	2nd expt	1st expt	2nd expt	1st expt	2nd expt	1st expt	2nd expt
I	+	−	−	3·3‡	10·0	40	20	0·2	1·2	0	0	—	Dead
II	+	+	−	2·5	6·0	50	20	0·02	0·2	0·1	0·1	—	—
III	+	+	−	1·5	6·0	60	150	0	0	7·5	21	—	—
IV	+	+	+	10	9·5	410	240	0	0	20	29	Medium	Strong
V	+	+	+	10	11·0	50	35	0	0	5	14	—	Mild
I				1·65		20		0·1		0			
+	+	+	−	+		+		+		+		—	
III				0·75		30		0		4			
I					5		10		0·6		0		
+	+	+	+		+		+		+		+		
IV					5		120				15		Mild

All recipients have been pretreated with Freund's complete adjuvant.

* Ionic strength in M NaCl equivalents: I = 0·007; II = 0·007–0·033; III = 0·034–0·053; IV = 0·054–0·15; V = 0·16–0·25.

† Immunoglobulins antigenically characterized (identical results for the two experiments).

‡ Units = volume (ml) × titre (reciprocal).

TABLE 6. Pathogenic capacity of fractions obtained by DEAE-cellulose chromatography of anti-autoantigen T sera

Pools*	Presence† of IgG2	IgG1	IgM	Injected volume (ml)	Total spermagglutination units‡ ($\times 10^{-2}$)	Total spermotoxic units ($\times 10^{-2}$)	Total C-fixing units ($\times 10^{-2}$)	AIAO in recipients
I	+	−	−	7·0	6·7	100·0	210·0	Medium
II	+	−	−	12·0	0·6	7·4	3·0	Dead
III	+	+	±	13·5	4·3	24·5	25·0	Mild
IV	+	+	±	5·5	1·0	0·3	0·06	—
V	+	+	+	7·5	1·1	0·6	0	—
VI	+	+	+	10·5	0·5	0·1	0	Mild

All recipients have been pretreated with Freund's complete adjuvant.

* Ionic strength in M NaCl equivalents: I = 0·007; II = 0.007–0·008; III = 0·008–0·0082; IV = 0·0085–0·13; V = 0·135–0·18; VI = 0·18–0·25.

† Immunoglobulins antigenically characterized (identical results for the two experiments).

‡ Volume (ml) × titre (reciprocal).

Fractionation of anti-T sera. As seen from Table 6, pool I, derived from DEAE cellulose chromatography of 80 ml of anti-T sera and eluted at the lowest ionic strength, was the only fraction which induced the disease. The lesions, essentially restricted to the epididymis, consisted of vascular permeability increase, oedema, dense invasion by PMN and mononuclear leucocytes together with some exfoliation of immature germinal cells from the testis (Figs 11, 12). At variance with this pool I, which contained only IgG2, pools III to VI, where other classes of immunoglobulin were present, induced only discrete lesions: hypospermogenesis and islets of mononuclear cells in the head and the tail of epididymis. Moreover, the sera from recipients of pool I and III demonstrated the presence of spermotoxic antibodies with titres of respectively 1:160 and 1:10.

In another experiment, the same partition of 45 ml of anti-T sera led to unsuccessful transfer of the disease. However, it was again the recipient of pool I, pure IgG2, that showed slight lesions: a few mononuclear cells extravasated in the epididymis head.

In addition to the fact that the two preceding experiments, involving chromatographic fractions, could be considered as reciprocal controls, other controls were included. No disease whatsoever happened in three recipients of 7·5–10 ml of normal sera and in seven other recipients of 5–15 ml of anti-ovalbumin globulins (see the Materials and Methods section). In the latter case, the efficiency of the transfer was attested by strong anaphylactic and Arthus reactions to intradermally injected ovalbumin.

DISCUSSION

The experiments described above essentially demonstrate that characteristic lesions of AIAO may be passively induced in guinea-pigs by antibodies directed against certain spermatozoa autoantigens and that this is mediated by restricted classes of antibodies; also, that the passive induction of lesions is facilitated by pretreatment of the recipients with FCA.

Passive lesions and lesions of actively induced AIAO. Do lesions passively induced by antisera represent an actual transfer of the actively induced disease? In the present experiments, the answer is yes with almost no doubts. The lesions are greatly similar in the two situations, with epididymal cellular infiltrations and vascular permeability increase and with germinal-cell exfoliation from seminiferous tubules. The only difference is the absence of cellular infiltration and of increased vascular permeability of the testis in the passively induced lesions. This latter fact is likely to be meaningful for the mechanism of AIAO.

A comparison of our data with those presented by other authors is made difficult by the differences in the pathological criteria used. Tung *et al.* (1971b) describe a moderate infiltration of PMN—most of them marginating along endothelial lining of vessels in rete testis and epididymis—that cannot be

considered as the passive transfer of the actively induced disease. Pokorna (1969, 1970), Willson *et al.* (1972) and Nagano & Okumura (1973) describe aspermatogenesis without cellular infiltration in the testis after passive transfer. Unfortunately, these authors, did not examine the epididymis which in our experience, is always the first site of lesions with the rete testis, in actively as well as passively induced AIAO.

Effective role of the injected antibodies. It has been observed in this laboratory that non-castrated male guinea-pigs immunized with autoantigens S or P and presenting lesions of AIAO, not only made anti-S or P, but also anti-T antibodies; while similarly treated castrated male guinea-pigs produced antibodies directed only against the immunizing autoantigen (Toullet & Voisin, 1974). Furthermore, it has been shown that anti-T spermotoxic and cytotoxic antibodies could be formed after i.d. injections of T autoantigen without adjuvants. We infer from these findings that an immune aggression directed against seminiferous cells may result in the release of sperm autoantigens and consequently lead to an active process of autoimmunization. Therefore one must be extremely careful before concluding that lesions have been passively induced. In particular, the appearance of lesions should be investigated early enough. The interval of 6–10 days between immune serum injection and pathological examination was chosen to verify that no important lesions resulting from active immunization had taken place (Toullet *et al.*, 1970; Voisin & Toullet, 1968). The choice of a longer interval, for example three weeks (Pokorna, 1970, Willson *et al.*, 1972) does not eliminate the possibility of an active immunization. On the other hand, the lesions must last for several days to consider that antibodies play an important role in the mechanism of the experimental disease. A disappearance of lesions in 1 or 2 days or even in a few hours (Tung *et al.*, 1971b) cannot be considered satisfying in this respect. Lesions described only at 24 and 48 hr (Nagano & Okumura, 1973) cannot be considered as equivalent of AIAO, even if they look like early actively-induced lesions. In the present experiments, incipient lesions, comparable in intensity to those described by Tung *et al.* (1971b) were noted as early as 1 day after serum injections and important lesions were noticed from day 6 to the end of the experiments, on day 13.

Responsible antigens. Three spermatozoa autoantigenic systems can induce lesions of AIAO when injected to guinea-pigs with FCA (Voisin & Toullet, 1968). While the histological pictures at the mature phase are identical, they differ at the beginning and the immunological features are clearly distinct, as well as the cytological localization of the corresponding antigens. P and T give rise to high amounts of antibodies of different classes, in particular complement-fixing IgG2. Only anti-T antibodies are spermotoxic and spermatidotoxic since only T antigen is on the cytoplasmic membrane. P- and T-induced lesions resemble Arthus reactions. S induces an intense-delayed hypersensitivity phenomenon and a restricted humoral response almost lacking IgG2 but rich in anaphylactic IgG1 antibodies; it also induces epididymal and testicular lesions with a microscopical aspect close to that of delayed hypersensitivity reactions (Toullet & Voisin, 1969 and 1974; Toullet *et al.*, 1970, Voisin & Toullet, 1968, 1969 and 1973).

The present observations are in keeping with the preceding results in that only anti-P and anti-T antibodies, but practically not anti-S, are able to induce passive lesions of AIAO.

Responsible antibodies. The problem of the responsible antibody class is as important as the preceding one and linked to it since different types of antigens induce preferentially certain classes of antibodies. This was studied by injecting a limited number of guinea-pigs with DEAE fractions of anti-T and anti-P immune sera. The role of anti-T pure IgG2 antibodies has been clearly demonstrated since the pathogenic activity was restricted to the most cationic fractions, shown to be pure IgG2 uncontaminated by either IgG1 or IgM. As for anti-P fractions, only the ones containing IgG1 anti-P antibodies, as well as IgG2 and IgM immunoglobulins but without detectable complement-fixing activity, were able to transfer the disease. Furthermore, anti-P IgM was detected neither in hyperimmune sera (sucrose gradient) nor in the active DEAE fractions (Sephadex G-200). Finally, it cannot be excluded that anti-P IgG2 activity might have been masked in an *in vitro* test by the strong competition with anti-IgG1. In view of that possibility, a co-operative action of IgG1 and IgG2 antibodies in the induction of the lesions is conceivable, in agreement with preceding studies on cutaneous (Maillard & Voisin, 1970) and glomerular lesions.

Another noticeable point is the considerable quantity of antibody required for a successful transfer: in spite of the dilution and metabolic degradation, the titre in serum recipients at the time autopsy, 6–13 days after serum injection, remained at a level far superior to that found in all animals showing an active AIAO after a single injection of antigen. The reason for this is not clearly understood. One possibility is that a massive but transitory administration of immune serum may be equated to a lower but sustained active production of antibodies. Alternatively, a special type of cytophilic antibody that would exist only in minute amounts in serum would be required, in conjunction with other types, to induce the lesions. It has been seen that reducing the dose by half lead to inactive DEAE fractions or mixtures of fractions.

Our present finding that hyperimmune sera are more efficient than sera of early immunization is not without possible controversies. First of all, early antisera contain much less antibodies than hyperimmune sera. This does not imply that early types of antibodies would not be more efficient if utilized in greater quantities. Two other reports deal with successful passive transfers of experimental autoimmune diseases with early antibodies: Willson *et al.* (1972) transferred guinea-pig testicular lesions with early (7 days) sera: Nakamura & Weigle (1969) transferred autoimmune rabbit thyroiditis with early sera (drawn between, day 4 and day 15 and injected in the same sequence) but not by late sera. One trial by us (not reported here) applying the same method to guinea-pig AIAO did not succeed. A report (Vladutiu & Rose, 1971) similar to our results, transferred thyroiditis into normal mice with late antibodies. Therefore, this problem is still open to discussion.

Action of FCA on recipients. Pretreating the recipients with FCA 1 or 2 weeks before injecting the antibodies greatly increases the incidence of successful transfers. Several possibilities might explain this action, such as increased vascular and tissular permeability or cellular activation, or liberation of a particular substance.

Increased vascular (Voisin, Toullet & Voisin, 1964) and tissular (Willson *et al.*, 1973) permeability is observed after injection of FCA and also in delayed hypersensitivity. This is a necessary condition to allow the action of antibodies in lesion induction, to take place. A preferential site for the crossing of the tissular barrier is the rete testis and vasa efferentia (Johnson, 1972, Setchell, 1974), but the tissular permeability may be extended to large areas of testicle by agents like cadmium—for globulins, in the rat (Gupta & Barnes, 1967) and FCA—for peroxidase, in the guinea-pig (Willson *et al*, 1973). Whatever the sequence of mechanisms, an increased vascular, tissular and tubular permeability is bound to favour the formation of antibody-induced lesions on antigen-bearing cells with resulting inflammatory consequences.

The increased rate of generation of effector cells in lymphoid organs, and the increased differentiation to an activated state (Mackaness & Blanden, 1967; Paraf, 1970) following FCA injection may play a key role, the exact nature of which is not clear (K cells? non-specifically activated macrophages?). One cannot exclude a role of a hypothetical substance (mediator?) liberated in the organism of FCA-injected animals. In any case, our data on the 'adjuvant' role of FCA in passive induction of testicular lesions are in agreement with those of other authors (Pokorna, 1969 and 1970; Willson *et al.*, 1972).

The FCA pretreatment may contribute to discriminate between the two pathogenic antigen-antibody systems (P and T) since the non-cytotoxic anti-P IgG1 plus IgG2? is more dependent on the help of FCA than the anti-T IgG2, spermotoxic and probably a better releaser of spermatic enzymes. As to the almost complete inability of the anti-S serum to transfer AIAO, it may indicate that cellular hypersensitivity is necessary for the induction of the disease (Brown *et al.*, 1967, Toullet & Voisin, 1969; Voisin & Toullet, 1969).

Mechanism of AIAO. The main interest of these studies is to bring some light to certain parts of the mechanism of AIAO and by extension, of autoimmune diseases. It is of importance to realize that three different antigens contained in one single cell, spermatozoon, when mixed with FCA may induce the active disease and that they may act through different mechanisms: delayed hypersensitivity, Arthus hypersensitivity and complement-dependent serocytotoxicity, the two last types of reactions being transferable by serum antibodies, for P and T antigens.

In effect, the autoimmune disease induced by injection of tissue, cells or even a crude-soluble extract from one particular organ may be considered as a compound of several immuno-pathologic responses, each being determined by one of the autoantigens contained in the immunizing material. Thus, the

mechanism of an experimental autoimmune lesion should be studied in the following order: a search for possible multiplicity of the responsible autoantigens; a purification (or at least a good separation) of the various autoantigens; separate and combined trials of the immunopathologic responses to these autoantigens, and finally, a study of the interactions of the immune reactions involved in the disease.

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