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A new murine model of autoimmune orchitis induced by immunization with viable syngeneic testicular germ cells alone. I. Immunological and histological studies

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SUMMARY

Experimental autoimmune orchitis (EAO) was produced in C3H/He mice with as high as 100% incidence by two or three s.c. injections of 1×10^7 viable syngeneic testicular germ cells (TC) without resorting to adjuvants, *Bordetella pertussis* vaccine, or other immunological manipulations. On day 40 after the first injection of TC, the lesions induced were characterized by interstitial infiltration of inflammatory cells and severe hypospermatogenesis in the testis with resulting whole organ atrophy and, in contrast, by a complete lack of epididymitis. Immunological studies revealed that this form of immunization caused both delayed-type hypersensitivity and humoral antibody responses to syngeneic TC. We compared the susceptibilities to the induction of this type of EAO among six different strains of inbred mice comprising A/J, AKR, BALB/c, C3H/He, C57BL/6 and DBA/2 mice. All strains except for DBA/2 mice developed lesions of EAO to a greater or lesser extent, and severe disease was induced with high frequency in two strains, C3H/He and A/J. As this murine model of EAO can be induced without the use of Freund's complete adjuvant and *B. pertussis* vaccine, it is simply 'autoimmune' in nature and may provide new ways for further investigation into the immunological mechanisms which regulate deleterious autoimmune reactions to germ cell antigens leading to the male infertility.

Keywords testes autoimmune orchitis male infertility delayed-type hypersensitivity autoantibodies

INTRODUCTION

Studies on immunopathogenesis and immunoregulation of experimental autoimmune orchitis (EAO) have been carried out using the guinea pig because of the ease of disease induction by s.c. injection with homologous testicular antigen emulsified in Freund's complete adjuvant (FCA) (Tung, Leong & McCarty, 1977; Hiramine & Hojo, 1984a, 1984b.; Hojo & Hiramine, 1982, 1985). For further investigation into these subjects the development of murine EAO model with a high incidence is indispensable, because the mouse has the distinct advantage of the availability of a wide range of immunological markers and a well-defined genetic background. Some investigators have reported the production of EAO in mice by immunization with testicular tissue homogenate emulsified in FCA plus a concomitant i.v. injection of pertussis vaccine (Bernard *et al.*, 1978; Sato, Hirokawa & Hatakeyama, 1981). We have encountered many difficulties in producing typical orchitic lesions in a variety of inbred mice following immunization with homologous testicular extract in FCA plus pertussis vaccine. At that time we were able to induce typical lesions only in a very limited number of mice studied (approximately 15%) despite elevated titres of circulating anti-sperm antibodies (Itoh *et al.*, 1989). More recently, Sakamoto *et al.* (1985) found that cyclophosphamide-pretreated mice that received a single s.c. injection with testicular cells, without using adjuvant, developed slight to mild EAO lesions and suggested that the mechanism that facilitated the EAO induction might be attributable to the elimination of endogenous suppressor cells by cyclophosphamide.

In the present experiments, we found that two or three s.c. injections with syngeneic testicular germ cells (TC) caused autoimmune orchitis with hypospermatogenesis in mice without any other manipulations, such as the use of adjuvants, *Bordetella pertussis* vaccine, immunopotentiating drugs, neonatal thymectomy, irradiation, chemical modification of the autoantigens, or artificial destruction of blood–testis barrier. Here we introduce the method which easily produces murine EAO of a better quality, and describe both the characteristic histopatho-

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logy of the disease and the cell-mediated and humoral immune responses to syngeneic TC.

MATERIALS AND METHODS

Mice

C3H/He mice were purchased from Shizuoka Laboratory Centre, Hamamatsu, Japan. Mice of strains A/J, BALB/c, C57BL/6, DBA/2 and AKR were purchased from Charles River Japan, Kanagawa, Japan.

Preparation of TC suspension

Testes were excised from syngeneic mice, teased with scissors into cold HBSS and passed through a stainless-steel mesh. The TC were harvested by centrifugation at 400 *g* for 10 min, washed three times in cold HBSS and adjusted to designated concentrations in HBSS after counting the viability using a trypan blue dye exclusion. The TC suspension contained more than 99% germ cells at all stages of spermatogenesis; the remainder (< 1%) were Sertoli cells and Leydig cells.

Experimental design

Male mice (aged 10–12 weeks) were divided into four groups as follows: group I, mice that received a single injection with 0·2 ml of HBSS alone; group II, mice that received a single s.c. injection with 1×10^7 TC in 0·2 ml of HBSS (single-dose regime); group III, mice that were injected twice at a 2-week interval with 1×10^7 TC (double-dose regime); group IV, mice that were injected three times at 2-week intervals with 1×10^7 TC (triple-dose regime). Each group consisted of 10–16 mice. On day 40 after the first immunization, all mice were anaesthetized, then exsanguinated through the axillary artery, and the blood was taken from individual mice to determine autoantibodies to TC in serum. Organs or tissues (testes, epididymides, thyroid glands, lungs, liver, kidneys, adrenal glands and pancreas) were removed for histological examinations. Delayed-type hypersensitivity (DTH) reaction to syngeneic TC was measured by delayed foot-pad reaction on day 40 after the first immunization.

Evaluation of delayed foot-pad reaction to syngeneic TC

Just before injection of test antigens for elicitation of DTH reaction, foot-pad thickness was measured with a dial-thickness gauge (micrometer, Mitutoyo, Tokyo, Japan). Then, 1×10^6 syngeneic TC in 50 μl of HBSS or 50 μl of HBSS alone were injected into the hind foot-pads on day 39 after the first immunization. After 24 h, the foot-pad thickness was measured with the gauge. The degree of reaction was expressed as the increased thickness, and 0·1 mm was expressed as 1 unit.

ELISA for autoantibodies to TC

Assay of antibodies to syngeneic TC was done in accordance with the ELISA procedure for detecting anti-sperm antibodies in mice with testicular autoimmunity (Itoh *et al.*, 1989). TC (10^5) in 100 μl of HBSS were added to each well of a 96-well microELISA plate (MS-3596F, Sumitomo Bakelite, Tokyo, Japan) precoated with poly-L-lysine (Sigma, St Louis, MO; 2 μg/100 μl per well). These were centrifuged at 400 *g* for 5 min. The adherent TC were fixed for 5 min in 0·1% glutaraldehyde. After the fixative was aspirated and washed three times with phosphate-buffered saline (PBS) supplemented with 0·05% Tween 20 (Wako Pure Chemical, Osaka, Japan) (PBS-T), 150 μl of PBS containing 1% bovine serum albumin (Sigma) were added to each well and kept overnight at 4°C to minimize non-specific background. After a wash with PBS-T, the plates were air-dried completely. The fixed TC on the microELISA plates were rehydrated in PBS and then 100 μl of methanol containing 0·3% H_2O_2 were added to each well in order to inactivate endogenous peroxidase activity contaminating the antigen layer. After 10 min, the fixed TC were washed three times with PBS-T.

Fifty microlitres of mouse serum samples serially diluted with 3% goat serum in PBS-T, each in duplicate, were added to the microELISA well and incubated for 2 h at room temperature. The wells were then washed five times in PBS-T, and incubated with 50 μl of horseradish-peroxidase-conjugated goat anti-mouse IgG + IgM (Jackson Immunoresearch Laboratories, West grove, PA; working dilution 1/5000) for 2 h at room temperature. After the wells were washed five times in PBS-T, 50 μl of *o*-phenylenediamine dihydrochloride (Wako) at 400 μg/ml, and 50 μl of H_2O_2 (0·04%) were added to each well. After 10 min, 50 μl of 1·0 N H_2SO_4 were added to each well, and the absorbance at 492 nm was determined with a micro-plate reader (MPR-A4, Toyo Soda, Tokyo, Japan).

Histological examination of testes and epididymides

The testes, epididymides and other organs were fixed in 10% phosphate-buffered formaldehyde (PH 7·2), embedded in paraffin and cut. The sections, 3 μm thick, were stained with haematoxylin and eosin. Pathology indices (PIs) were determined on each testis and epididymis. The orchitis or epididymitis were graded on a 0–4 scale as follows: 0, no morphological change; 1, scarce perivascular inflammatory cell infiltration; 2, several interstitial foci of inflammatory cells; 3, extensive inflammatory cell infiltration beneath the tunica albuginea that involves the interstitium and several seminiferous tubules in testis or moderate inflammatory cell infiltration in epididymis; and 4, inflammation that replaces 50% or more of the whole area of testis section. Hypospermatogenesis in testis was graded, on a 0–4 scale, using the degree of percentage of the seminiferous tubules showing disappearance of mature germ cells, desquamation of germinal epithelium and giant cell formation in the prepared section: 0, 0%; 1, 1–5%; 2, 6–25%; 3, 26–50%; and 4, 51–100%.

RESULTS

Histopathology of testes and epididymides in C3H/He mice

PIs of orchitis, hypospermatogenesis and epididymitis in each group are summarized in Fig. 1. No pathological changes were detected in testes of TC-untreated mice (group I) (Fig. 2a). In mice that received a single injection with TC (group II), most areas of the testis sections were almost completely free of impairment of spermatogenesis, but, occasionally, foci of mild hyperplasia of Leydig cells and slightly damaged seminiferous tubules were observed. In mice injected twice (group III), inflammatory cell responses in the interstitium just beneath the tunica albuginea and in the rete testis were developed (Fig. 2b). Moderate hypospermatogenesis always followed this type of autoimmune orchitis. Desquamation of the germinal epithelium and formation of multi-nuclear giant cells within the seminiferous tubules were observed in mice injected twice (Fig. 2c). Three TC injections (group IV) resulted in severe atrophy of the testes.

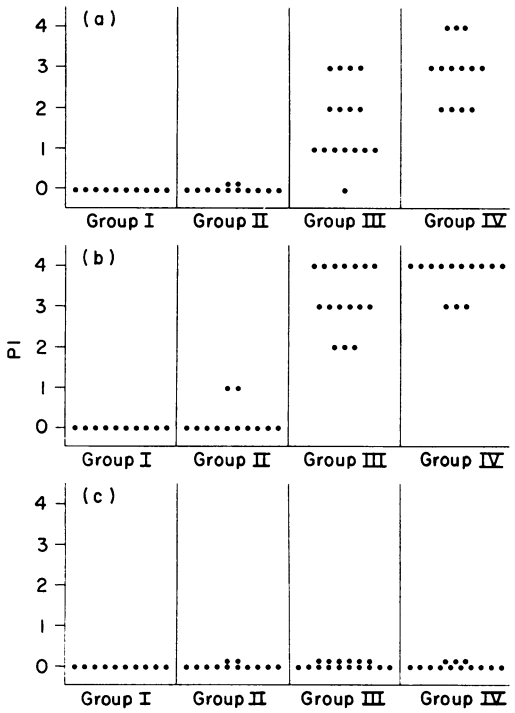

Fig. 1. Histopathological findings in the testes and epididymides in groups of C3H/He mice. (a) Pathological index (PI) of orchitis; (b) PI of hypospermatogenesis; (c) PI of epididymitis. Group I, untreated; group II, single s.c. injection of testicular germ cells (TC); group III, two injections of TC; group IV, three injections of TC.

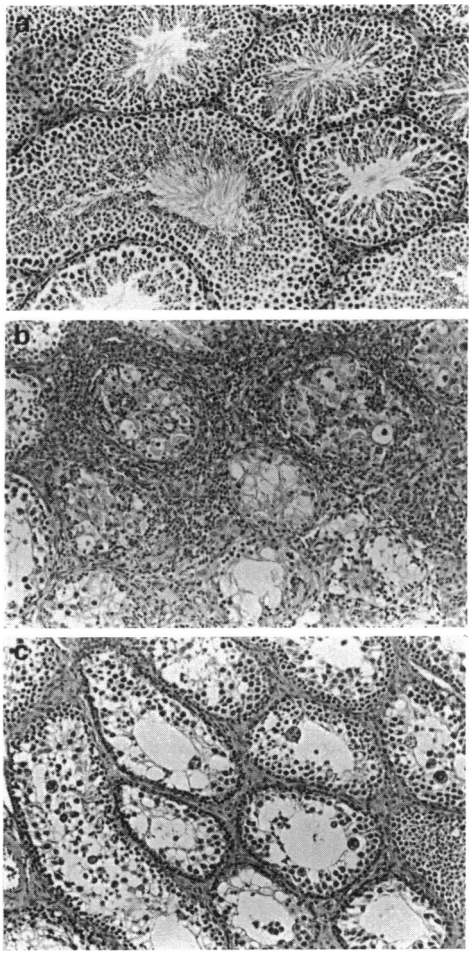

Fig. 2. Comparison of histological picture of testis between a C3H/He mouse untreated with testicular germ cells (TC) and a mouse receiving two doses of TC. (a) A section of testis of a TC-untreated mouse (group I). Note closely packed, intact seminiferous tubules containing all stages of maturation of the germinal epithelium from spermatogonia to spermatozoa; (b) a section of testis of a mouse that received two TC-doses (group III). Note inflammatory cell infiltration in the thickened intertubular interstitium and devastation of seminiferous tubules leading to hypospermatogenesis; (c) a section of testis of a mouse of group III. Note marked depression of spermatogenesis with multinuclear giant cell formation. Magnification ×90.

The lesions were characterized by extensive interstitial infiltration of inflammatory cells beneath the tunica albuginea and severe hypospermatogenesis in testes. In contrast, neither inflammatory cell infiltration in the interstitium of the epididymides nor degenerative change of the epididymal duct epithelia was evident in all three groups of TC-injected mice, except for decreased number of spermatozoa in the epididymal duct lumens of animals receiving the repeated injections, probably resulting from the aspermatogenesis in the testis (Fig. 3). In addition, no lesions could be detectable in organs or tissues other than testis in TC-injected mice.

Delayed foot-pad reactions to syngeneic TC

The degrees of delayed foot-pad reaction to syngeneic TC in each group of C3H/He mice examined on day 39 after the first TC injection (mean ± s.d.) were as follows: group I, 1·0 ± 0·5 U; group II, 2·2 ± 1·2 U; group III, 3·4 ± 1·3 U; and Group IV, 5·3 ± 1·4 U (Fig. 4). A significant increase in the foot-pad reaction was observed in mice that received a single injection with TC compared with TC-untreated mice ($P < 0·02$). With the increasing times of TC injection, the levels of the reaction to TC became higher (group II *versus* group III, $P < 0·05$; group III *versus* group IV, $P < 0·001$). In all groups, no increase of more than 0·5 U in the foot-pad thickness was observed at 24 h after injection with 50 μl of HBSS alone (data not shown).

Anti-TC autoantibodies in sera

Figure 5 shows the level of circulating autoantibodies to TC in each group of C3H/He mice determined as absorbance (492 nm) read at serum dilution of 1/800. Since the absorbance values obtained from normal virgin female mouse sera at this dilution were lower than 0·150, this value was considered as a border line. The absorbance values (mean ± s.d.) of anti-TC antibodies in each group were as follows: group I, 0·184 ± 0·059; group II, 0·295 ± 0·102; group III, 0·399 ± 0·134; group IV, 0·464 ± 0·168; and the group of normal virgin female mice, 0·124 ± 0·020. In TC-untreated male mice (group I) the absorbance values were very low, although significantly high compared with normal virgin female mice ($P < 0·01$). The level of anti-TC antibodies in the group II mice was higher than that of the untreated mice (group I) ($P < 0·01$). In group III mice there was no significant rise in the autoantibody level compared with group II mice ($P < 0·1$). However, in group IV mice the autoantibody level was significantly higher than that in group II mice ($P < 0·025$).

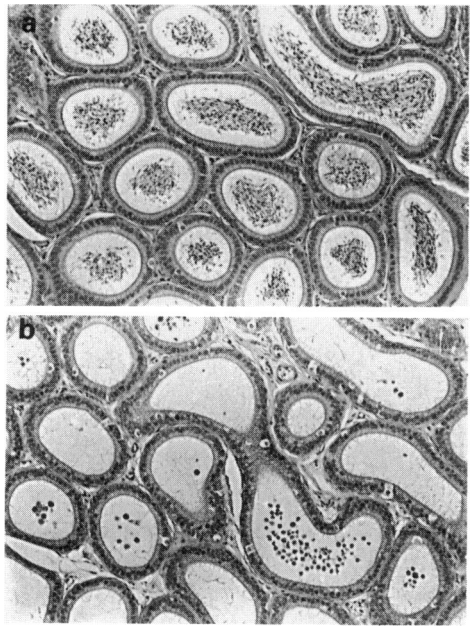

Fig. 3. Comparison of histological picture of epididymis between a C3H/He mouse untreated with testicular germ cells (TC) and a mouse receiving three doses of TC. (a) Epididymis of a TC-untreated mouse (group I). Note many normal sperm in the ducts; (b) epididymis of a mouse immunized three times with TC (group IV). Although a markedly decreased number of spermatozoa together with degenerating germ cells were present in the lumens, there is no evidence of inflammation in the interstitium and of degenerative changes of the epididymal duct epitheliums. Magnification × 90.

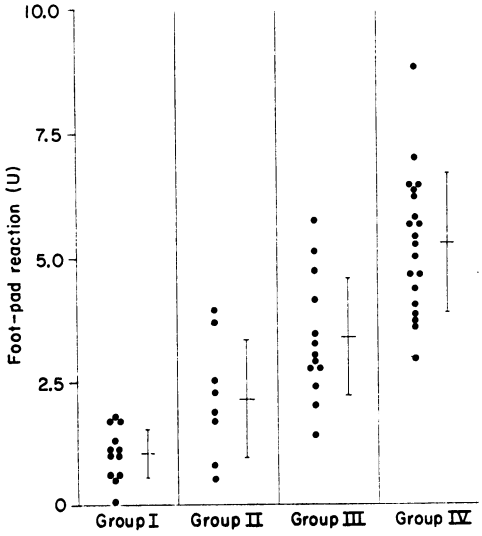

Fig. 4. Delayed foot-pad reaction to syngeneic testicular germ cells (TC) in various groups of male C3H/He mice: group I, untreated mice; group II, single s.c. injection of TC; group III, two doses of TC; group IV, three doses of TC. Vertical bars represent s.d.; 0·1 mm of increased foot-pad swelling is expressed as 1 U.

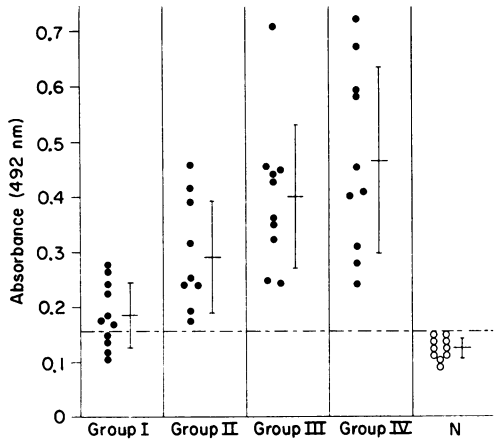

Fig. 5. Autoantibody response to testicular germ cells (TC) in groups of C3H/He mice: group I, untreated mice; group II, single s.c. injection of TC; group III, two doses of TC; group IV, three doses of TC; and N, normal female virgin mice. All serum specimens were assayed at serum dilution of 1/800. The broken line shows the cut-off limit of the assay. Vertical bars represent s.d.

Table 1. Incidence and severity of experimental autoimmune orchitis (EAO) in different strains of mice

		Group III		Group IV	
Strain	H-2	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
C3H/He	k	16/16 (100)	7/16 (44)	13/13 (100)	10/13 (78)
A/J	a	9/9 (100)	5/9 (56)	4/4 (100)	3/4 (75)
C57BL/6	b	2/6 (33)	0/6 (0)	1/6 (17)	0/6 (0)
AKR	k	2/6 (33)	1/6 (17)	0/6 (0)	0/6 (0)
BALB/c	d	0/6 (0)	0/6 (0)	2/6 (33)	0/6 (0)
DBA/2	d	0/6 (0)	0/6 (0)	0/6 (0)	0/6 (0)

Incidence, number of mice exhibiting pathological index (PI) of 1 or more in orchitis and/or PI of 2 or more in hypospermatogenesis/total number of mice studied.

Severity, number of mice exhibiting PI of 4 in orchitis and/or PI of 4 in hypospermatogenesis/total number of mice studied.

Group III mice received two injections of 1×10^7 testicular germ cells (TC) at a 2-week interval; group IV mice received three injections of 1×10^7 TC at 2-week intervals. See Materials and Methods for PIs.

Incidence and severity of EAO in different strains of mice induced by repeated injections with TC

Susceptibility to the induction of EAO was compared among six different strains of inbred mice, including A/J, AKR, BALB/c, C3H/He, C57BL/6 and DBA/2. Mice were injected with TC twice (group III) or three times (group IV). The incidence and severity of testicular lesions are summarized in Table 1. To avoid the possibility that a focal hypospermatogenesis (PI = 1) may be produced artificially during preparation of testis sections, the sections showing PI of 1 or more in orchitis and/or 2 or more in hypospermatogenesis are interpreted as significant lesions. It

was found that five of the strains of mice (all except for DBA/2) developed testicular lesions in varying degrees and that severe EAO was induced with a high frequency not only in C3H/He mice but also in A/J mice. In all A/J mice exhibiting PI of 4, the whole area of testis sections was replaced by severe hypospermatogenesis and diffuse inflammation. In C57BL/6 strain, orchitis (PI = 1) developed in two of six mice receiving two TC doses and in one of six mice receiving the three doses. In AKR strain, although only two of six AKR mice that were given two doses developed testicular lesions, one of them showed severe hypospermatogenesis (PI = 4) with mild orchitis (PI = 2) followed by marked atrophy of the testes. However, none of the six mice receiving three doses of TC developed significant lesions. In BALB/c strain, two doses of TC did not cause significant pathological changes, but three TC doses did cause hypospermatogenesis (PI = 3) with slight orchitis (PI = 1) in two of them. In the DBA/2 strain, none of the mice that received two or three doses of TC gave rise to any significant pathological lesions. Interestingly, in all six strains of mice studied, epididymitis was observed only in one A/J mouse that developed a slight perivascular inflammation in the interstitium without affecting epididymal ducts (PI = 1 of epididymitis) after three TC doses.

DISCUSSION

We have demonstrated that viable syngeneic TC, when injected subcutaneously without using any adjuvants, are highly immunogenic, enough to induce both cellular and humoral immune responses to TC antigens even in male mice given only a single dose of TC; further, two or more injections of TC into appropriate strains of mice enabled them to induce a definite EAO with surprisingly high incidence. Therefore, it can be stated that the secondary immune response to TC is quite essential to the induction of this form of EAO. The efficacy of live TC for induction of EAO is documented by an additional experiment in which repeated injection with glutaraldehyde-pretreated TC failed to induce EAO (Itoh *et al.*, in preparation). Therefore, injection with viable but not dead TC is important for induction of orchitis. Production of EAO in mice has been reported in several laboratories. The 'classic' form of murine EAO is ordinarily induced by active immunization with testicular tissue homogenate emulsified in FCA plus a concomitant use of pertussis vaccine (Bernard *et al.*, 1978; Sato *et al.*, 1981). Hargis, Malkiel & Berkelhammer (1968) were the first to obtain aspermatogenesis in mice immunized with a mixture of testicular tissue homogenate and *B. pertussis* vaccine without the use of FCA. However, this form of EAO was characterized by disturbances in spermatogenesis but no accumulations of mononuclear cells. The employment of FCA and *B. pertussis* has proved to be instrumental in altering the completeness of blood–testis barrier indirectly, as well as in augmenting of DTH reaction, with resulting inflammatory cell response (Pelletier, Nemirovsky & Hugon, 1981; Sewell *et al.*, 1986; Adekunle *et al.*, 1987). Moreover, it is possible that immunization using a whole testis homogenate elicites not only immune responses against the germ cells, but also the responses against other testicular components such as Leydig cells, Sertoli cells and basal lamina of the seminiferous tubules (Sato *et al.*, 1981; Ichinohasama, Hirokawa & Hatakeyama, 1986). Another study of murine EAO model showed that C3H/He mice given cyclophosphamide 2 days prior to a single s.c. dose of TC (1×10^7 cells) developed a delayed foot-pad response to TC and mild EAO lesions, whereas no such effects were seen in TC-dosed but cyclophosphamide-untreated mice (Sakamoto *et al.*, 1985); these investigators thought that pretreatment with cyclophosphamide, probably via inhibiting immunoregulatory mechanisms that normally exist prior to TC immunization, could abrogate the unreponsiveness to TC, resulting in EAO induction.

However, in about 20–30% of susceptible strain of mice that were thymectomized on day 3, a mild orchitis occurred along with other organ-specific autoimmune diseases such as thyroiditis and gastritis (Taguchi & Nishizuka, 1981). It is noteworthy that in the post-thymectomy orchitis, epididymitis consistently occurs prior to the development of orchitis. Using indirect immunofluorescence, it was demonstrated that autoantibodies against germ cells detected in sera of the thymectomized mice exclusively bound to acrosomes of mature sperm, but not to spermatids. It seems, therefore, that the testicular autoimmunity following thymectomy on day 3 may be directed predominantly to acrosomal proteins of mature sperm within epididymal ducts, rather than to immature germ cells within seminiferous tubules, consequently giving a marked preference for epididymitis. In contrast, the EAO model presented here is unique in its striking lack of epididymitis, and preponderance of orchitis with aspermatogenesis. Therefore, differing from post-thymectomy orchitis, the immune responses involved in this model may be mainly directed to antigens on immature testicular germ cells but not to antigens on epididymal spermatozoa bearing late developing male antigens. The antigenic determinants on TC for stimulating the autoreactive clones are not yet identified, but we suppose that earlier developing male antigens on TC, which diminish or disappear as germ cells differentiate to mature sperm, may be chiefly responsible for the induction of this EAO model.

Among the six different inbred strains examined, C3H/He (H-2^k) and A/J (H-2^a) strains were most susceptible to this EAO. AKR mice, another H-2^k strain, were less susceptible to the disease induction than C3H/He (H-2^k) mice. This finding suggests that a genetic susceptibility associated with genes outside the H-2 haplotype may contribute to the development of this EAO. In our laboratory, failure to induce both classic EAO and post-thymectomy orchitis in C3H/He mice was observed (unpublished). Failure of C3HB/FeJ mice to develop the classic EAO and of C3H/HeJ mice to develop post-thymectomy orchitis have also been reported (Kojima & Prehn, 1981; Teuscher *et al.*, 1985). Thus, mice of C3H strains may be resistant to induction of the classic EAO and the post-thymectomy orchitis. This implies that our EAO model may be put on a genetic basis distinct from that for induction of the classic or post-thymectomy orchitis.

Adoptive transfer experiments by the immune cells, and an establishment of line cells for induction of the EAO in C3H/He mice are now in progress for investigating the precise immunopathological mechanisms for this model of testicular autoimmunity. So far we have found that both cellular ($CD4^+$ T cells) and humoral (IgG- and IgA-secreting plasma cells) immunity participate in immunohistochemical manifestation of the orchitic lesion (Itoh *et al.*, manuscript in preparation). In the study on immunological testis lesions, which are often found in human azoospermia, this murine EAO may constitute a powerful

experimental model for the analysis of pathogenesis of the disease.

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REFERENCES

ADEKUNLE, A.O., HICKEY, W.F., SMITH, S.M., TUNG, K.S.K. & TEUSCHER, C. (1987) Experimental allergic orchitis in mice.IV. Preliminary characterization of the major murine testis specific aspermatogenic autoantigen(s). *J. reprod. Immunol.* **12,** 49.

BERNARD, C.C.A., MITCHELL, G.F., LEYDON, J. & BARGERBOS, A. (1978) Experimental autoimmune orchitis in T-cell-deficient mice. *Int. Arch. Allergy appl. Immunol.* **56,** 256.

HARGIS, B.T., MALKIEL, S. & BERKELHAMMER, J. (1968) Immunologically induced aspermatogenesis in the white mouse. *J. Immunol.* **101,** 374.

HIRAMINE, C. & HOJO, K. (1984a) Adoptive transfer of suppression of experimental allergic orchitis with lymphoid cells from antigen-pretreated guinea pig. *Clin. Immunol. Immunopathol.* **30,** 202.

HIRAMINE, C. & HOJO, K. (1984b) Requirement of B lymphocytes in local adoptive transfer of experimental allergic orchitis by lymph node cells. *Am. J. reprod. Immunol.* **6,** 85.

HOJO, K. & HIRAMINE, C. (1982) Suppression of experimental allergic orchitis and cellular immune responses in guinea pig by pretreatment with testis antigen in incomplete Freund's adjuvant. *Int. Arch. Allergy appl. Immunol.* **69,** 40.

HOJO, K. & HIRAMINE, C. (1985) In vivo effects of cyclosporin A: abrogation of the induction of experimental allergic orchitis and sparing of the generation of suppressor cells. *Int. Arch. Allergy appl. Immunol.* **78,** 63.

ICHINOHASAMA, R., HIROKAWA, K. & HATAKEYAMA, S. (1986) Spermatogenic disturbance induced in mice by combined local injection of monoclonal antibodies to Sertoli cell and to basal lamina of seminiferous tubule. *Am. J. reprod. Immunol. Microbiol.* **10,** 158.

ITOH, M., HIRAMINE, C., KOSETO, M. & HOJO, K. (1989) Enzyme-linked immunosorbent assay (ELISA) for detecting antisperm antibodies in mice with testicular autoimmunity. *Am. J. reprod. Immunol.* **21,** 9.

KOJIMA, A. & PREHN, R.T. (1981) Genetic susceptibility to post-thymectomy autoimmune diseases in mice. *Immunogenetics*, **14,** 15.

PELLETIER, R.M., NEMIROVSKY, R.C. & HUGON, J.S. (1981) Effects of immunization with Freund's complete adjuvant and isologous spermatozoa on the seminiferous epithelium and blood-testis barrier in guinea pigs. *Anat. Rec.* **199,** 197.

SAKAMOTO, Y., HIMENO, K., SANUI, H., YOSHIDA, S. & NOMOTO, K. (1985) Experimental allergic orchitis in mice. I. A new model induced by immunization without adjuvants. *Clin. Immunol. Immunopathol.* **37,** 360.

SATO, K., HIROKAWA, K. & HATAKEYAMA, S. (1981) Experimental allergic orchitis in mice. Histopathological and immunological studies. *Virchows Arch. [Pathol. Anat.]* **392,** 147.

SEWELL, W.A., MOERLOOSE, P.A., MCKIMM-BRESCHKIN, J.L. & VADAS, M.A. (1986) Pertussigen enhances antigen-driven interferon-γ production by sensitized lymphoid cells. *Cell. Immunol.* **97,** 238.

TAGUCHI, O. & NISHIZUKA, Y. (1981) Experimental autoimmune orchitis after neonatal thymectomy in the mouse. *Clin. exp. Immunol.* **46,** 425.

TEUSCHER, C., SMITH, S.M., GOLDEBERG, E.H., SHEARER, G.M. & TUNG, K.S.K. (1985) Experimental allergic orchitis in mice. I.Genetic control of susceptibility and resistance to induction of autoimmune orchitis. *Immunogenetics*, **22,** 323.

TUNG, K.S.K., LEONG, C. & MCCARTY, T. (1977) Pathogenesis of experimental allergic orchitis. III. T lymphocyte requirement in local adoptive transfer by peritoneal exudate cells. *J. Immunol.* **118,** 1774.