

Studies of $Sl/SI^d \leftrightarrow +/+$ mouse aggregation chimaeras

II. Effect of the steel locus on spermatogenesis

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Summary

Mutant mice of Sl/SI^d genotype are deficient in melanocytes, erythrocytes, mast cells and germ cells. Deficiency of melanocytes, erythrocytes and mast cells is not attributable to an intrinsic defect in their precursor cells but to a defect in the tissue environment that is necessary for migration, proliferation and/or differentiation. We investigated the mechanism of germ cell deficiency in male Sl/SI^d mice by producing aggregation chimaeras from Sl/SI^d and $+/+$ embryos. Chimaeric mice with apparent white stripes were obtained. Two of four such chimaeras were fertile and the phenotypes of resulting progenies showed that some Sl/SI^d germ cells had differentiated into functioning sperms in the testis of the chimaeras. In cross sections of the testes of chimaeras, both differentiated and nondifferentiated tubules were ob-

served. However, the proportions of type A spermatogonia to Sertoli cells in both types of tubules were comparable to the values observed in differentiated tubules of normal $+/+$ mice. We reconstructed the whole length of four tubules from serial sections. Differentiated and nondifferentiated segments alternated in a single tubule. The shortest differentiated segment contained about 180 Sertoli cells and the shortest nondifferentiated segment about 150 Sertoli cells. These results suggest that Sertoli cells of either Sl/SI^d or $+/+$ genotype make discrete patches and that differentiation of type A spermatogonia does not occur in patches of Sl/SI^d Sertoli cells.

Key words: aggregation chimaera, spermatogenesis, Sertoli cell, steel locus, tissue environment, patch size.

Introduction

A double gene dose of mutant alleles at either the W locus (chromosome 5) or the Sl locus (chromosome 10) is known to produce the pleiotropic effects of sterility, hypoplastic anaemia and depletion of mast cells and melanocytes (Russell, 1979; Kitamura *et al.* 1985). The underlying mechanisms of the depletion of erythrocytes, mast cells and melanocytes have been investigated by transplantation of cells and tissues. In spite of the similarity in the phenotypic expression of W/W^v and Sl/SI^d mice, the underlying mechanisms are quite different. Depletion of these three types of cells in W/W^v mice is attributable to a defect in their precursor cells. In fact, neural crest cells of $+/+$

embryos can differentiate into melanocytes in the skin of W/W^v embryos (Mayer & Green, 1968; Mayer, 1970) and the transplantation of bone marrow cells from congenic $+/+$ mice normalizes the number of erythrocytes (Russell *et al.* 1959; McCulloch *et al.* 1964; Russell & Bernstein, 1968) and mast cells (Kitamura *et al.* 1978) in W/W^v recipients.

In contrast, the depletion of erythrocytes, mast cells and melanocytes in Sl/SI^d mice is due to a defect in the tissue environment. The neural-crest of normal $+/+$ embryos failed to produce melanocytes when combined with Sl/SI^d embryonic skin, whereas neural crest from Sl/SI^d embryos produced melanocytes when combined with $+/+$ neural-crest-free embryonic skin (Mayer & Green, 1968; Mayer, 1970). The

bone marrow of Sl/SI^d mice contained normal precursors of erythrocytes and mast cells, but transplantation of $+/+$ bone marrow cells does not cure the anaemia and mast-cell depletion of Sl/SI^d mice (McCulloch *et al.* 1965; Bernstein, 1970; Kitamura & Go, 1979).

Although the sterility of W/W^v and Sl/SI^d mice is known to result from the absence of germ cells in the gonads, the process has not been analysed by transplantation techniques such as those used for investigations of melanocytes, erythrocytes and mast cells (Hall, 1983). From histological investigations of homozygous or double heterozygous W and Sl embryos, Bennett (1956) and Mintz & Russell (1957) claimed an interference with the migration of the primordial germ cells (PGCs). On the other hand, McCoshen & McCallion (1975) did not find such interference and thought that the reduced number of PGCs in Sl/SI^d embryos is caused either by a failure in proliferation or by an excessive rate of cell death.

As it is apparently difficult to inject PGCs into embryos, we used aggregation chimaeras to clarify the mechanism of germ cell depletion in testes of Sl/SI^d mice. Normal numbers of type A spermatogonia were observed in seminiferous tubules of $Sl/SI^d \leftrightarrow +/+$ chimaeras and normal differentiation of Sl/SI^d germ cells was confirmed by analysing the genotypes of offspring. However, when the whole seminiferous tubule of a $Sl/SI^d \leftrightarrow +/+$ chimaera was reconstructed from serial histological sections, segments with and without differentiated germ cells occurred alternately. Since numbers of type A spermatogonia were normal even in segments without differentiated germ cells (type B spermatogonia to spermatids), we attributed the differentiation arrest of such segments to a defective microenvironment composed of Sertoli cells of the Sl/SI^d genotype.

Materials and methods

Mice

C57BL/6- $SI^d/+$ and WB- $Sl/+$ mice as well as their F_1 hybrids (hereafter WBB6 F_1) were raised in our laboratory. The original stocks were derived from the Jackson Laboratory, Bar Harbor, Maine. C3H/He (hereafter C3H) and ICR (albinos employed as foster mothers of aggregated embryos) mice were purchased from the Shizuoka Laboratory Animal Center, Hamamatsu, Japan. The C3H strain is A/A (agouti), and the WB and C57BL/6 strains a/a (nonagouti).

Aggregation chimaeras

The method described by Mintz *et al.* (1973) and by Hoppe & Pitts (1973) was used with a slight modification. Briefly, 8- to 16-cell embryos were aggregated in Hoppe's medium containing phytohaemagglutinin (Difco Laboratories, Detroit, Michigan) after removal of the zona pellucida with

acidified Tyrode's solution (pH 2.5; Nicolson *et al.* 1975). Subsequently, the aggregated embryos were cultured at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. The resulting chimaeric blastocysts were transferred to the uterus of a 3.5-day *post coitum* pseudopregnant ICR female mouse.

Test mating

Male chimaeras with apparent white stripes, which were considered to be $(Sl/SI^d;a/a) \leftrightarrow (+/+;A/A)$ chimaeras (Nakayama *et al.* 1988), were kept with three virgin WB- $+/+;a/a$ mice in a cage. Female mice that became pregnant were replaced by other virgin mice. Each pregnant female was kept in an individual cage to determine the genotypes of the offspring. These genotypes were identified by the coat colour; nonagouti hairs with dilution indicated $Sl/+;a/a$ or $SI^d/+;a/a$, nonagouti hairs without dilution $+/+;a/a$, and agouti hairs without dilution $+/+;A/A$.

Diameter of seminiferous tubules and counting of cells

Testes of chimaeras were removed, fixed in Bouin's solution and embedded in paraffin. Sections (6 μ m thick) were stained with haematoxylin and eosin. Diameters of seminiferous tubules were measured with the micrometer attached to an eyepiece of the microscope. The circumference of the tubule was calculated by assuming the cross section of the tubule to be a circle. Numbers of Sertoli cells and type A spermatogonia were counted under the microscope, and proportions of type A spermatogonia to Sertoli cells were obtained.

Reconstruction of seminiferous tubules

Serial sections of a testis of a $(Sl/SI^d;a/a) \leftrightarrow (+/+;A/A)$ chimaera were stained with haematoxylin and eosin. Low-power micrographs of all serial sections were printed. Each seminiferous tubule was followed in the micrographs and differentiation of germ cells was scored under the microscope. Each cross section of the tubules was classified into the following three categories; (1) differentiated tubules, in which numerous spermatids were observed, (2) nondifferentiated tubules, in which only type A spermatogonia were detectable, and (3) intermediate tubules, in which both of the above-mentioned features were observed in a single cross section.

Electron microscopy

Under ether anaesthesia, the testes of a chimaeric mouse (no. 2) were fixed by perfusion through the thoracic aorta. The fixative consisted of a mixture of 2.5% glutaraldehyde and 1% formaldehyde at pH 7.2 with cacodylate buffer. After perfusion, the testes were removed, cut into small pieces and fixed in the same fixative for 2 h at room temperature. After rinsing, specimens were postfixed with cacodylate-buffered 1% OsO_4 and embedded in Epon 812 through ethanol solutions and propylene oxide. Block staining was carried out in 50% ethanol containing 0.1% uranyl acetate. Thick sections were stained with toluidine blue for light microscopy and thin sections were stained with uranyl acetate followed by lead citrate for electron microscopy.

Table 1. Testis weights, fertility and genotype of offspring of putative ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) male chimaeras that were mated with WB- $+/+;a/a$ female mice

Mice chimaera no.	Weight of testis (mg)		Number of offspring of each genotype					
			$Sl/+;a/a$ or $Sl^d/+;a/a^*$		$+/+;a/a$		$+/+;A/a$	
	Right	Left	Male	Female	Male	Female	Male	Female
1	51	40	38†	27	0	0	13†	8
2	62	56	35†	31	0	0	2†	6
3	18	18	0	0	0	0	0	0
4	76	42	0	0	0	0	0	0

* $Sl/+;a/a$ and $Sl^d/+;a/a$ were not distinguishable in the present genetic background.

† The presence of male progenies indicated that germ cells of both $Sl/Sl^d;a/a$ and $+/+;A/A$ genotypes had the Y chromosome.

Results

Since mice of the Sl/Sl^d genotype are sterile, WB- $Sl/+;a/a$ and C57BL/6- $Sl^d/+;a/a$ mice were mated to obtain $Sl/Sl^d;a/a$ embryos. The resulting embryos were aggregated with C3H- $+/+;A/A$ embryos. In addition to $Sl/Sl^d;a/a$ embryos, embryos obtained by the present cross contained three other genotypes (i.e. $Sl/+;a/a$, $Sl^d/+;a/a$ and $+/+;a/a$). However, since only $Sl/Sl^d;a/a$ animals are nonpigmented, four healthy male mice with apparent white stripes were assumed to represent ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaeras (Nakayama *et al.* 1988) and used for further study.

Each ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaera was kept with three female WB- $+/+;a/a$ mice for 12 weeks in a single cage. Two of four chimaeras were fertile, whereas the remaining two chimaeras were sterile (Table 1). Although both testes of chimaera no. 3 were small, the testes of chimaera no. 4 were normal in weight. Moreover, we could not find any morphological abnormalities in the genitalia of chimaera no. 4, and the cause of the sterility of this chimaera remained unclear.

86 and 74 offsprings were obtained from chimaeras nos 1 and 2, respectively (Table 1). The phenotypes of progenies include nonagouti dilute mice (considered to be either $Sl/+;a/a$ or $Sl^d/+;a/a$) and agouti ($+/+;A/A$) mice. None of the offspring had nonagouti hairs without dilution (i.e. were $+/+;a/a$), indicating that the testes of such chimaeras contain only $Sl/Sl^d;a/a$ and $+/+;A/A$ spermatogonia. This confirmed that the male mice with apparent white stripes were ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaeras. Moreover, this result clearly demonstrated that spermatogonia of $Sl/Sl^d;a/a$ genotype differentiated to sperms in the testes of ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaeras.

Testes of four ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaeras were investigated histologically. Testes of nonchimaeric WBB6F₁- $+/+$ and Sl/Sl^d mice were also investigated as controls. Although chimaeras nos 1 and 2 were fertile and chimaeras no. 3 and 4 were sterile, the histological features of all testes were similar. Differentiated, nondifferentiated and intermediate seminiferous tubules were observed (Fig. 1, Table 2). Numbers of these three types of tubules were counted in the largest cross section. The proportion of nondifferentiated tubules was greatest in the right testis of chimaera no. 3 and smallest in the right testis of chimaera no. 4 (Table 2).

The circumference of seminiferous tubules and the number of Sertoli cells per cross section of tubules were determined in testes of ($Sl/Sl^d;a/a$) \leftrightarrow ($+/+;A/A$) chimaeras and nonchimaeric WBB6F₁- $+/+$ and Sl/Sl^d mice. The circumference of seminiferous tubules was significantly greater but the number of Sertoli cells per cross section was significantly less in WBB6F₁- $+/+$ mice than in WBB6F₁- Sl/Sl^d mice (Table 3). As a result, numbers of Sertoli cells per unit length of tubule circumference were less in WBB6F₁- $+/+$ mice than in WBB6F₁- Sl/Sl^d mice. In testes of chimaeras, numbers of Sertoli cells per unit length of tubule circumference were less in differentiated tubules than in nondifferentiated tubules (Table 3). Sertoli cells appeared to be 'diluted' by differentiating germ cells both in seminiferous tubules of WBB6F₁- $+/+$ mice and in differentiated tubules of chimaeras.

The circumference of seminiferous tubules and the number of Sertoli cells per cross section were influenced by differentiation of germ cells. However, absolute number of Sertoli cells is considered to be constant in adult animals, since Sertoli cells do not divide in the adult testes (Steinberger & Steinberger, 1971). Since it was rather difficult to assess the absolute numbers of Sertoli cells and type A spermatogonia in the whole testis, we determined proportions of type A spermatogonia to Sertoli cells in

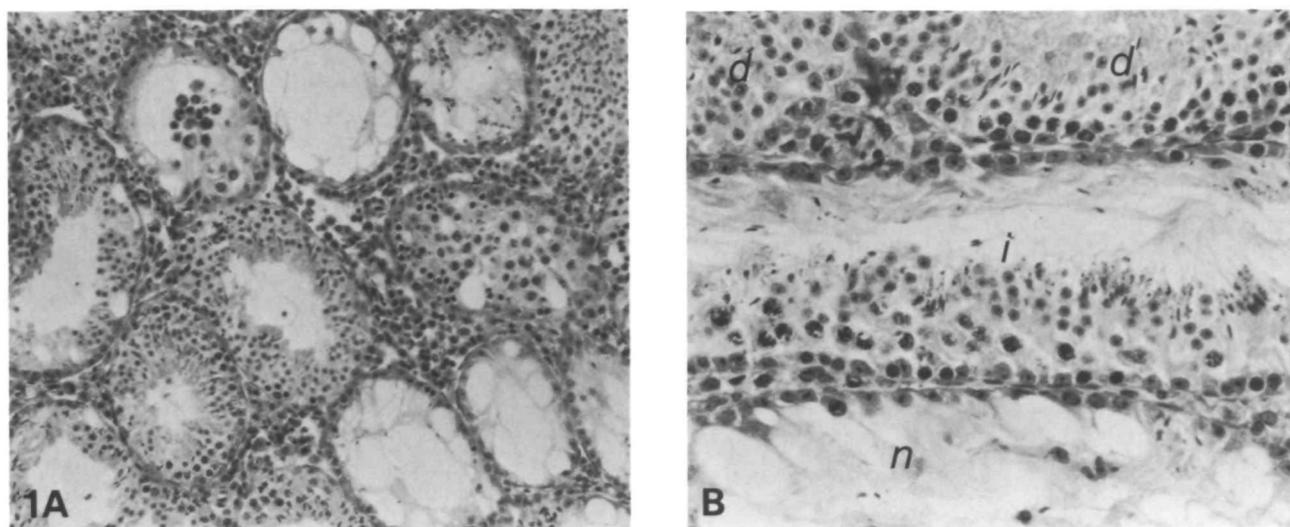


Fig. 1. (A) Light micrograph of a paraffin section of the testis of chimaera no. 1 showing three types of tubules; (1) differentiated tubules, in which numerous spermatids were observed, (2) nondifferentiated tubules, in which only type A spermatogonia were detectable and (3) intermediate tubules, in which both of the above-mentioned features were observed in a single section. $\times 115$. (B) A higher magnification of three typical types of tubules of the chimaera. *d*, differentiated; *i*, intermediate and *n*, nondifferentiated tubule(s). H and E $\times 230$.

Table 2. Numbers of differentiated, nondifferentiated and intermediate tubules in cross sections of testes in nonchimaeric $WBB6F_{1-}/+/+$ and Sl/Sl^d mice and $(Sl/Sl^d;a/a)\leftrightarrow(+/+; A/A)$ chimaeras*

Mice	Right testis				Left testis			
	Weight (mg)	No. of tubules			Weight (mg)	No. of tubules		
		Differentiated	Intermediate	Nondifferentiated		Differentiated	Intermediate	Nondifferentiated
$WBB6F_{1-}/+/+$ no. 1	130	128	0	0	126	127	0	0
$WBB6F_{1-}/+/+$ no. 2	123	108	0	0	127	101	0	0
$WBB6F_{1-}/+/+$ no. 3	119	88	0	0	121	119	0	0
$WBB6F_{1-}Sl/Sl^d$ no. 1	12	0	0	84	12	0	0	97
$WBB6F_{1-}Sl/Sl^d$ no. 2	14	0	0	98	14	0	0	132
$WBB6F_{1-}Sl/Sl^d$ no. 3	14	0	0	127	13	0	0	99
Chimaera no. 1	51	92	5	12	40	82	9	32
Chimaera no. 3	18	8	14	88	18	14	4	82
Chimaera no. 4	76	78	2	1	42	65	3	47

* Testes of chimaera no. 2 were used for electron microscopy.

Table 3. Circumference of seminiferous tubules and number of Sertoli cells per cross section of seminiferous tubules in testes of nonchimaeric $WBB6F_{1-}/+/+$ and Sl/Sl^d mice and $(Sl/Sl^d;a/a)\leftrightarrow(+/+; A/A)$ chimaeras

Mice	Type of tubules	No. of Sertoli cells per cross section of tubule	Circumference of tubule (mm)	No. of Sertoli cell per 1 mm circumference
$WBB6F_{1-}/+/+$	Differentiated	$14.9 \pm 0.8^*$	$0.669 \pm 0.020^*$	$21 \pm 0.66^*$
$WBB6F_{1-}Sl/Sl^d$	Nondifferentiated	$22.3 \pm 1.4^{*\ddagger}$	$0.308 \pm 0.012^{*\ddagger}$	$72 \pm 1.95^{*\ddagger}$
Chimaera	Differentiated	$15.3 \pm 0.6^\dagger$	$0.556 \pm 0.015^\dagger$	$27 \pm 0.49^\dagger$
	Nondifferentiated	$16.6 \pm 0.7^\dagger$	$0.317 \pm 0.013^\dagger$	$55 \pm 2.64^\dagger$

* Mean \pm s.e. of 10 tubules randomly selected from three mice.

† Mean \pm s.e. of 10 segments randomly selected from chimaera no. 1.

‡ $P < 0.05$, when compared to the corresponding differentiated tubules by *t*-test.

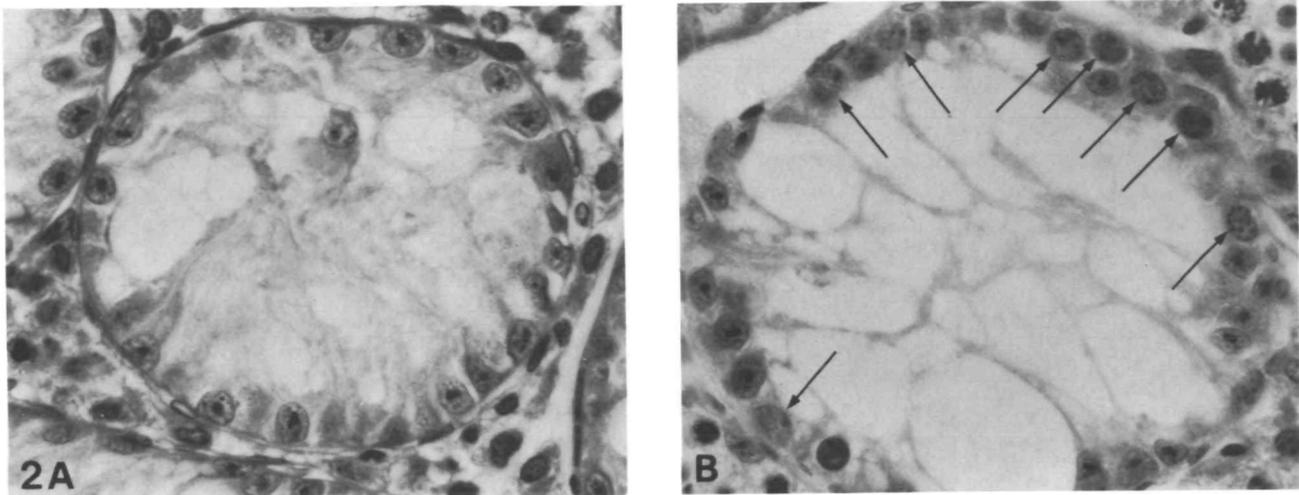


Fig. 2. Light micrographs of paraffin sections showing an aspermatogenic seminiferous tubule of WBB6F₁-Sl/St^d (A) and a nondifferentiated tubule of the chimaera no. 1 (B). Many type A spermatogonia (arrows) are observed in the nondifferentiated tubule of chimaera no. 1. H and E ×460.

Table 4. Proportion of type A spermatogonia to Sertoli cells in seminiferous tubules of nonchimaeric WBB6F₁-+/+ and Sl/St^d mice and (Sl/St^d;a/a)↔(+/+;A/A) chimaeras

Mice	Proportion of type A spermatogonia to Sertoli cells	
	In differentiated tubules	In nondifferentiated tubules
WBB6F ₁ -+/+ no. 1	135/420 (32 %)	—
WBB6F ₁ -+/+ no. 2	121/409 (30 %)	—
WBB6F ₁ -+/+ no. 3	134/457 (29 %)	—
WBBF6 ₁ -Sl/St ^d no. 1	—	61/1205 (5 %)
WBBF6 ₁ -Sl/St ^d no. 2	—	54/1328 (4 %)
WBBF6 ₁ -Sl/St ^d no. 3	—	35/1024 (3 %)
Chimaera no. 1	180/583 (31 %)	192/688 (28 %)
Chimaera no 2*	118/406 (29 %)	120/408 (29 %)
Chimaera no. 3	111/415 (27 %)	131/474 (29 %)
Chimaera no. 4	71/241 (29 %)	142/512 (27 %)

* Counted by using thick sections of the Epon-embedded specimen.

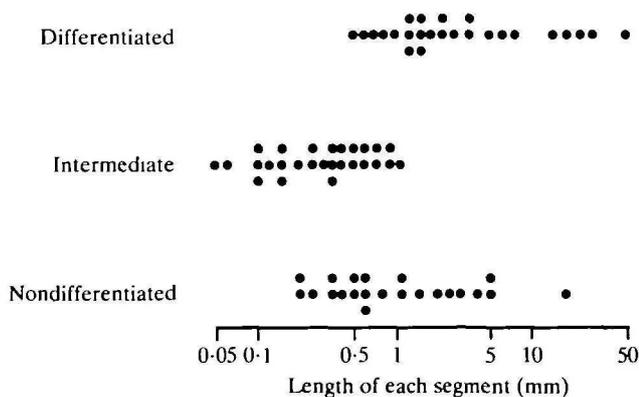


Fig. 3. Length of differentiated, intermediate and nondifferentiated segments. Data from four reconstructed tubules.

testes of (Sl/St^d;a/a)↔(+/+;A/A) chimaeras and nonchimaeric WBB6F₁-+/+ and Sl/St^d mice. The proportion of type A spermatogonia to Sertoli cells was significantly reduced in testes of WBB6F₁-Sl/St^d mice but not in testes of (Sl/St^d;a/a)↔(+/+;A/A) chimaeras (Fig. 2, Table 4). Even in nondifferentiated tubules of chimaeras, the proportion of type A spermatogonia was comparable to the proportion observed in tubules of WBB6F₁-+/+ mice.

Although proportions of type A spermatogonia to Sertoli cells were comparable in all examined tubules of (Sl/St^d;a/a)↔(+/+;A/A) chimaeras, differentiation occurred in some tubules but not in others. Reconstruction of the whole seminiferous tubules from serial sections was done to examine whether differentiated tubules were connected with nondifferentiated tubules. Four tubules in the right testis of

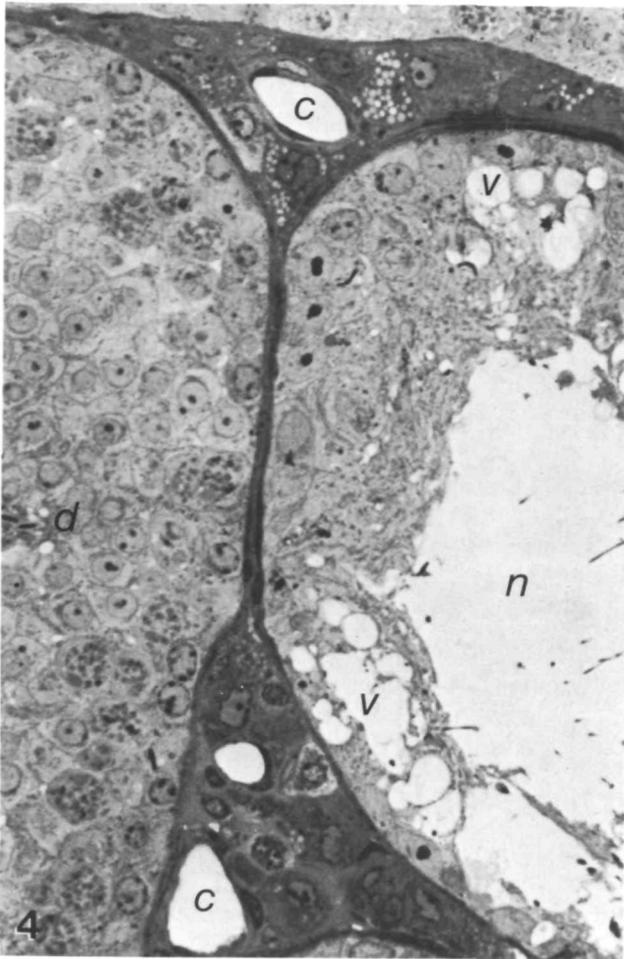


Fig. 4. Light micrograph of a plastic section showing differentiated (*d*) and nondifferentiated (*n*) tubules of chimaera no. 2. Many vacuoles (*v*) are seen in the Sertoli cells in the nondifferentiated tubule. Note clusters of Leydig cells near the nondifferentiated tubule. *c*, capillaries. $\times 550$.

chimaera no. 1 were reconstructed; segments with and without differentiated germ cells alternated with each other in a single tubule.

The length of differentiated segments varied from $500\ \mu\text{m}$ to $5\ \text{cm}$, and nondifferentiated segments from $200\ \mu\text{m}$ to $1.7\ \text{cm}$ (Fig. 3). In addition to the number of Sertoli cells per cross section (Table 3), we also counted the number of Sertoli cells in longitudinal sections of differentiated and nondifferentiated segments. There were 15.3 Sertoli cells in a cross section and 24.0 Sertoli cells per mm in a longitudinal section of the differentiated segment. In contrast, there were 16.6 Sertoli cells in a cross section and 44.2 Sertoli cells per mm in a longitudinal section of the nondifferentiated segment (all values are an average of 10 different segments). Therefore, the shortest differentiated segment contained about 180 Sertoli cells and the shortest nondifferentiated segment about 150

Sertoli cells. In differentiated segments of reconstructed tubules, spermatogenesis appears to be normal from histological observations.

Morphological study of Epon-embedded specimens was carried out to examine whether Sertoli cells of nondifferentiated segments were abnormal. First, thick sections of the testes of chimaera no. 2 were examined with the light microscope. As shown in Fig. 4, Sertoli cells and spermatogonia rested on the basal lamina in the nondifferentiated tubules. The basal lamina was not even in thickness but formed knobs in some parts. No apparent changes of Leydig cells were found, but the Leydig cells near the nondifferentiated tubules appeared to make a larger cluster than those near the differentiated tubules (Fig. 4).

With the electron microscope, many vacuoles were found in the cytoplasm of Sertoli cells of the nondifferentiated segment (Fig. 5). The junctional complexes between adjacent Sertoli cells were normal; they consisted of tight and gap junctions, and actin filament bundles were situated between the junctional plasma membrane and the endoplasmic reticulum (Fig. 5). Cytoplasmic organelles including mitochondria, smooth and rough varieties of endoplasmic reticulum and lysosomes appeared to be normal. The nucleolus in the nucleus of Sertoli cells showed a characteristic feature consisting of a central and two spherical bodies generally observed in laboratory rodents (Fawcett, 1975). Bundles of intermediate-sized filaments were frequently observed in the basal part of Sertoli cells (Fig. 6). Normal-shaped spermatogonia with an elliptic nucleus were detected.

In the intermediate tubule, various stages of degeneration were found in addition to normally differentiating germ cells. Pyknotic nuclei of spermatocytes and spermatids were prominent. Some of the elongated spermatids had an irregularly shaped head with an acrosome (Fig. 7). The orientation of the spermatids to the Sertoli cell was disorganized in the seminiferous epithelium.

Discussion

We produced four ($Sl/SI^d; a/a$) \leftrightarrow ($+/+; A/A$) aggregation chimaeras. Two of them were fertile when mated with $+/+; a/a$ females. The phenotypes of their offspring indicated that some of them were derived from $Sl/SI^d; a/a$ spermatogonia. To our knowledge, this is the first demonstration that spermatogonia of the Sl/SI^d genotype can be made to differentiate into functioning sperms.

Proportions of type A spermatogonia to Sertoli cells in seminiferous tubules of ($Sl/SI^d; a/a$) \leftrightarrow ($+/+; A/A$) chimaeras were comparable to the values observed in the tubules of WBB6F₁- $+/+$ mice. If

numbers of Sertoli cells per whole testis are assumed to be comparable between the chimaeras and WBB6F₁-+/+ mice, the absolute numbers of type A spermatogonia are comparable between them. This suggests that *Sl/Sl^d* PGCs migrate to gonads and differentiate into sperms if an appropriate tissue environment is present. Moreover, since mesenchymal cells of both *Sl/Sl^d;a/a* and *+/+;A/A* genotypes undoubtedly occur between the yolk sac and the gonads of chimaeric embryos, it is likely that PGCs migrate in a tissue environment composed of such a mixed cell population.

When histologically examined, differentiation of type A spermatogonia did not occur in some tubules of chimaeras. Differentiated and nondifferentiated tubules were adjacent in cross sections and, moreover, differentiated and nondifferentiated segments alternated in individual reconstructed tubules. Therefore, the differentiation arrest of type A spermatogonia may be attributable to a defect in the intratubular microenvironment. This is consistent with the result of Nishimune *et al.* (1980, 1984, 1985) who investigated the effect of the steel locus on

spermatogenesis by using WB-*Sl/+* and C57BL/6-*Sl^d/+* mice. Nishimune *et al.* (1980) made testes of both WB-*Sl/+* and *+/+* mice cryptorchid, and the cryptorchidism was subsequently reversed. They found that the differentiation in the *Sl/+* testes was apparently impaired at two steps, i.e. the differentiation of spermatogonia type A to type B and the meiotic division. Then, they cultured *in vitro* fragments of testes of C57BL/6-*Sl^d/+* and *+/+* mice that had been made cryptorchid. In testis fragments from C57BL/6-*+/+* mice, type A spermatogonia showed effective differentiation in response to an increased concentration of bovine serum (Nishimune *et al.* 1984) or fetuin (Nishimune *et al.* 1985). In testis fragments of C57BL/6-*Sl^d/+* mice, however, type A spermatogonia showed only limited differentiation even in the highest concentration of either bovine serum (Nishimune *et al.* 1984) or fetuin (Nishimune *et al.* 1985).

Two types of cells constructing seminiferous tubules may influence differentiation of germ cells, (1) Sertoli cells and (2) peritubular myoid cells (Tung & Fritz, 1987). From the present results, we cannot

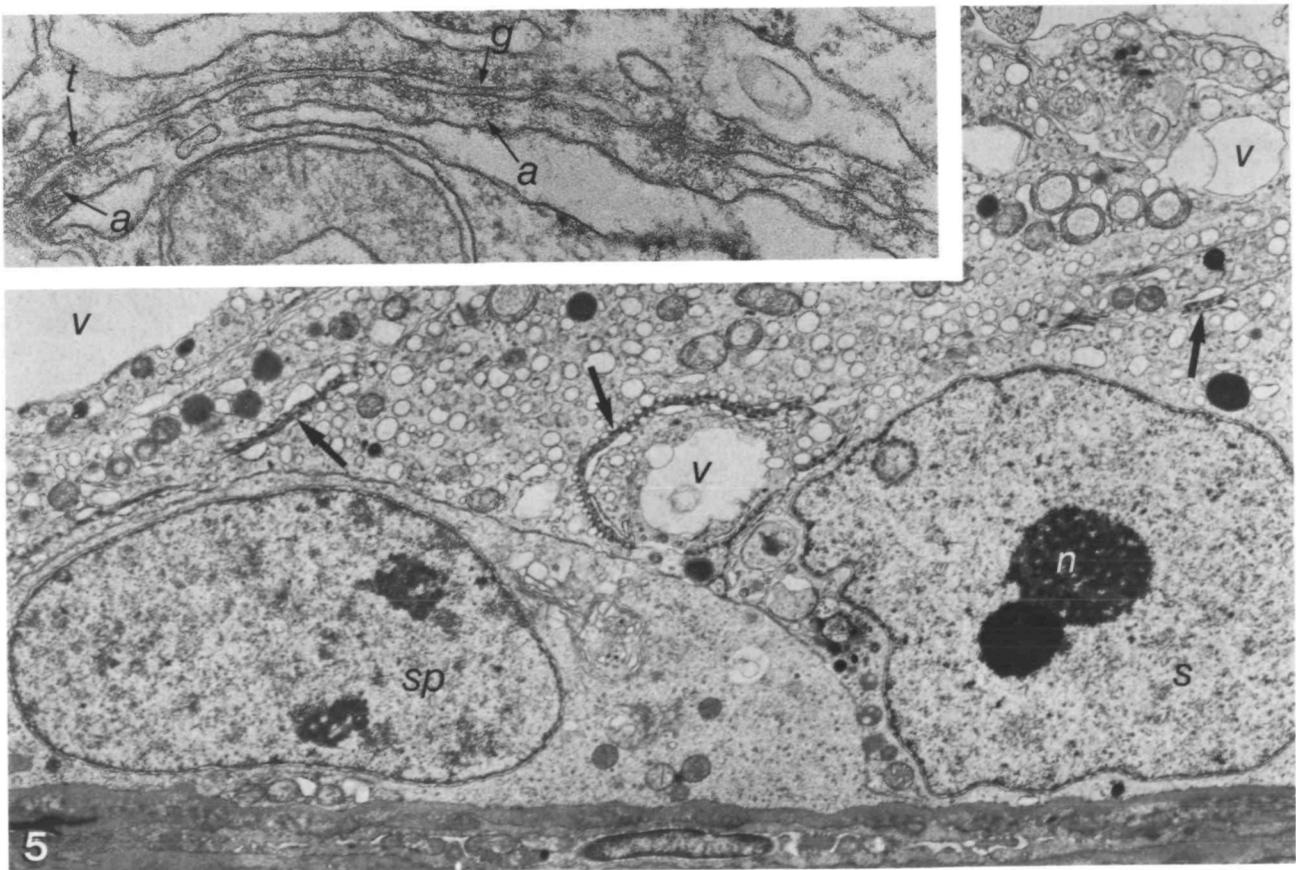


Fig. 5. Electron micrograph showing a spermatogonium (*sp*) and Sertoli cell (*s*) in the nondifferentiated tubule. Vacuoles (*v*) of various sizes and junctional complexes (arrows) between the Sertoli cells are found. The inset is a higher magnification of the junction consisting of tight (*t*) and gap (*g*) junctional regions with actin filament bundles (*a*). *n*, nucleolus $\times 7300$. Inset; $\times 72000$.

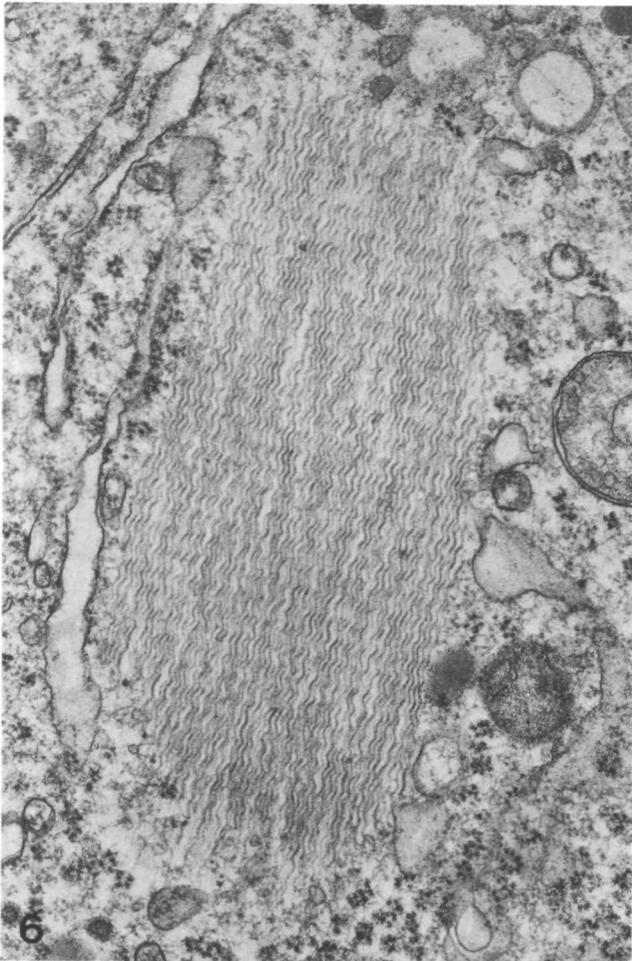


Fig. 6. A bundle of intermediate-sized filaments in the cytoplasm of the Sertoli cell in the nondifferentiated tubule. The filaments run parallel to each other with a zigzag course. $\times 33\,000$.

discriminate which of these plays the more important role in differentiation arrest of germ cells in seminiferous tubules of $(Sl/SI^d;a/a) \leftrightarrow (+/+;A/A)$ chimaeras. However, we incline towards the hypothesis that nondifferentiated segments of chimaeric tubules are composed of $Sl/SI^d;a/a$ Sertoli cells for the following reason. We recently analysed the skin of $Sl/SI^d \leftrightarrow +/+$ chimaeras and our results suggest that epithelial cells produce larger patches than underlying mesenchymal cells (Nakayama *et al.* 1988). In seminiferous tubules, Sertoli cells have an epithelial arrangement, whereas peritubular myoid cells have a mesenchymal arrangement. The present reconstruction study of tubules showed that about 180 and 150 Sertoli cells were present in the shortest differentiated and nondifferentiated segments, respectively. Therefore, we speculate that differentiated and nondifferentiated segments represent patches of Sertoli cells rather than the smaller patches of peritubular



Fig. 7. Deformed spermatid with many indentations in the cytoplasm of the Sertoli cell in the intermediate tubule. $\times 13\,000$.

myoid cells. Differentiated segments may be composed of $+/+;A/A$ Sertoli cells and nondifferentiated segments of $Sl/SI^d;a/a$ Sertoli cells. There is a possibility that Sertoli cells in each differentiated or nondifferentiated segment may have a clonal origin, as suggested by Mintz (1970, 1971) in the case of coat colour patches.

Some electron microscopical changes were detectable in Sertoli cells of nondifferentiated segments of $(Sl/SI^d;a/a) \leftrightarrow (+/+;A/A)$ chimaeras. Many vacuoles were present; bundles of intermediate-sized filaments appeared, and the knob formation of basal lamina was observed. However, these changes are not specific to the steel mutation but have been reported in various aspermatogenic conditions, such as in the testes of W^m/W^v mice, another aspermatogenic mutant (Nagano, 1977), autoimmune aspermatogenesis (Nagano & Okamura, 1973; Pelletier *et al.* 1981) and organ-cultured tubules from the cryptorchid testes (Nagano *et al.* 1984). The appearance of bundles of intermediate-sized filaments also occurs in inactive seminiferous tubules (Dym, 1974; Toyama, 1975; Nagano *et al.* 1984). In spite of these nonspecific changes, junctional complexes between Sertoli cells were maintained, suggesting that the blood–testis barrier may function to some extent.

In some areas of intermediate segments, degeneration of germ cells at various stages and normal

differentiation of germ cells are observed at the same time, suggesting that Sl/Sl^d and $+/+$ Sertoli cells are intermingled.

Individual Leydig cells appeared to be normal in the testes of $(Sl/Sl^d;a/a) \leftrightarrow (+/+;A/A)$ chimaeras. However, it appeared that Leydig cells near the nondifferentiated tubules made larger clusters than those near the differentiated tubules. Therefore, the testes of the chimaeras may be useful for examining the hypothesis proposed by Aoki & Fawcett (1978) that Leydig cells receive local feedback regulations from adjacent tubules.

From the above results, $Sl/Sl^d \leftrightarrow +/+$ aggregation chimaeras may be a useful tool for investigating the morphogenesis and the function of the testis.

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