The effects of a $t$-allele ($t^{AE5}$) in the mouse on the lymphoid system and reproduction

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SUMMARY

Mice homozygous for $t^{AE5}$, a recessive allele at the complex $T$-locus, are characterized by their unique short-tailed phenotype as well as by runting and low fertility. Histological and histochemical studies of the lymphoid and reproductive systems disclosed structural changes in the mutant spleen resembling those found in autoimmune conditions. Involution of the mutant thymus was greatly accelerated compared to normal. Necrotic changes occurred during spermiogenesis whereas ovarian structure was normal in mutants. The possible mechanisms of the mutant effects are discussed in the framework of other similar syndromes and the mode of action of alleles at the complex $T$-locus.

INTRODUCTION

A recessive allele at the complex $T$-locus, $t^{AE5}$, with a unique homozygous phenotype, was identified several years ago. $t^{AE5}$ homozygotes are viable with short tails, whereas homozygotes for any of the previously described recessive $t$-alleles are either embryonic lethals or, if viable, have normal tails. In addition, $t^{AE5}$ homozygotes are runted with characteristically poor growth, viability and fertility.

The syndrome of runting associated with low fertility, as well as preliminary findings of morphological thymus deficiencies in $t^{AE5}$ homozygotes, suggested a possible autoimmune nature of the mutant condition. This possibility is especially intriguing because of suspected involvement of the $T$-locus in the control of cell surface properties (Gluecksohn-Waelsch & Erickson, 1970) and the reports of serological identification of $t$-associated cell surface components with antigenic properties (Artzt & Bennett, 1975).

The studies reported here focus on the detection of histological and histochemical changes in the lymphoid system and gonads of $t^{AE5}$ homozygotes. It was hoped that such analysis might identify abnormalities in this particular

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mutant which would fit in with the proposed role of the T-locus in determining cell surface antigens.

MATERIALS AND METHODS

The $t^{AE5}$ mutation arose in the mouse colony at Albert Einstein College of Medicine in the phocomelic strain which itself was derived from a strain carrying the viable recessive allele $t^{11}$ (Dunn & Gluecksohn-Waelsch, 1953). In the course of breeding experiments with the phocomelic mutation, animals were found with an exceptional short-tailed phenotype. These bred true for short-tailedness and were considered to be homozygous for a newly arisen recessive t-allele, $t^{AE6}$. For the histological analysis, 25 homozygous adult males (2–12 months old) and 17 females (newborn to 12 months old) were used. Mice from a variety of inbred strains served as controls.

Organs to be examined were fixed in Carnoy’s, embedded in paraffin, and sectioned. Parallel sections of lymphoid organs, spleen and thymus, were stained with haematoxylin-eosin, toluidine-blue (pH 2-0) or alcian blue. Parallel sections of testes were stained with haematoxylin-eosin and Schiff reagent (Feulgen nuclear reaction), and sections of ovaries were stained with haematoxylin-eosin only.

Activity of spermatogenesis was measured by determining the average frequency of tubules with maturing spermatozoa (Oakberg, 1956) in left and right testes. After preliminary evaluation of sections from the central part of both testes of two males in the first series, 13–15 pseudoserial sections from both testes including about 2000 tubules were studied in the second series of five males. The absence of appreciable variation in the state of spermatogenesis between centre and periphery indicated that the poor activity in mutant mice was uniform and not of focal nature. Therefore, only 3–4 sections from peripheral and central parts of each testis were evaluated in the remaining two series.

Sperm was stripped into normal saline from the vasa deferentia of four $t^{AE5}/t^{AE5}$ males (3½–10 months old) and three control males (2–7 months old). Motility was scored qualitatively on a scale of 0 to ++++. Viability was assessed with the vital stain brilliant cresyl blue. Dry smears were prepared for morphological study. The morphology of 200 sperm from each male was studied with phase microscopy.

RESULTS

Breeding data

As mentioned earlier, $t^{AE5}$ homozygotes breed true for short-tailedness and produce tailless ($T/t^{AE5}$) and normal-tailed (+/$t^{AE5}$) offspring when outcrossed to Brachyury mice ($T/+). Breeding data are summarized in Table 1. The small litter size in experiment 1 is a reflexion of the poor breeding performance of $t^{AE5}$ homozygotes.
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Table 1

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Parents</th>
<th>No. of matings</th>
<th>Total no.</th>
<th>Tailless</th>
<th>Short tail</th>
<th>Normal tail</th>
<th>Litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$t^{AE5}/t^{AE5} \times t^{AE5}/t^{AE5}$</td>
<td>449</td>
<td>1557</td>
<td>0</td>
<td>1557</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>$t^{AE5}/t^{AE5} \times T/+ $</td>
<td>12</td>
<td>79</td>
<td>36</td>
<td>0</td>
<td>43</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Viability

$t^{AE5}$ homozygotes show drastically reduced viability at all ages. The small litter size indicates possible prenatal death. In addition, in one experiment, 205 newborn out of a total of 331 were found to die within the first 48 h of life. Survivors did not all reach weaning age. They appeared to be more susceptible to slight infections than either wild-type mice or other mutants.

Weight

The average weight of homozygotes at weaning was 8 g compared to 15–20 g for normal weanlings.

Histological results

Structural changes in the spleen

In mutant animals, the spleen could not be distinguished from normal (Fig. 1) for the first two months of life; there was a clear distinction of the red and white pulp with remnants of erythrophoiesis and a gradual appearance of distinct lymphoid follicles. In later stages the following abnormalities became noticeable in mutant mice: the incidence of megakaryocytes increased and signs of activation became visible in the white pulp, e.g. germinal centres and distinct perifollicular zones. Subsequently, a decline of activation as well as a decrease of the white pulp area became apparent (Fig. 2). There seemed to be an increase of proliferation of reticulum cells in the perifollicular zones, and later in the entire spleen. The observed morphological changes resembled those resulting from long-lasting antigenic stimulation which is first reflected in increased activation, later in proliferation of reticulum cells.

The proliferation of reticulum cells with increasing age results in the loss of the normal spleen architecture, i.e. in the reduction of the white and replacement of the red pulp, events known to be associated with a decline of functional capacity.

Structural changes in the thymus

The disintegration of cortical lymphocytes in the thymus of neonatal mutant mice of both sexes presented a conspicuous difference from the thymus of normal mice of several inbred strains. Nuclear debris and occasionally haematoxylin bodies were observed in newborn mutants (Fig. 3). At the age of 1–4
Fig. 1. Normal spleen of 10-month-old mouse with distinct white pulp (W) and residual erythropoiesis in the red pulp (E). Haematoxylin-eosin (HE), ×100.

Fig. 2. Spleen of a mutant 11-month-old female; follicles of the white pulp (W). Note increased incidence of reticulum cells (R) in the perifollicular zones. HE, ×100.

Fig. 3. Thymus of mutant newborn female; numerous remnants of disintegrated nuclei in the cortex, occasionally with formation of haematoxylin bodies (arrows). HE, ×700.

Fig. 4. Normal thymus of 2-month-old mouse with clear distinction of cortex (C) and medulla (M). HE, ×220.

Fig. 5. Thymus of a 9-month-old mutant mouse with focus of mast cells (MC) in parenchyma. Toluidine blue, at low pH. ×250.

Fig. 6. Pycnotic nuclei of mutant spermatogenic cells. Note swelling and vacuolization (arrows). HE, ×700.
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months the development of the mutant organ was indistinguishable from normal (Fig. 4), with a cortex thick and well distinguishable from the medulla in which pseudo-Hassal's bodies were found in some cases.

In 3 to 4-month-old mutant mice occasional mast cells were observed in the parenchyma; this cell type is absent at this age from the thymus parenchyma of normal mice of various inbred strains (Viklický, 1967). After the age of 6 months a change in the structure of the mutant organ was reflected in the reduction of the relative extent of the cortex and an increase in the incidence of mast cells (Fig. 5) which are more numerous in mutants than in control animals of the same age. The change of the cortex: medulla ratio in mutant mice is similar to that observed in the normal thymus during physiological involution, but in most cases it is much more extensive. In several such mice, the cortex was virtually absent. This advanced involution of the thymus in mutant mice may account for the fact that in many cases dissection failed to provide any thymic tissue for histology but only hypertrophic parathymic lymphatic nodes.

Gonads

Reproductive performance is unusually poor in both male and female \( t^{AE5} \) homozygotes. This is expressed in failure of many such animals to reproduce at all, in abnormally small litter size, high frequency of still-born young and premature termination of reproductive ability in breeding animals. For all these reasons, it is very difficult to maintain the stock, and frequent outcrosses become necessary.

Testicular histology and epididymal sperm

Table 2 summarizes the results of examination of testes of 25 homozygous mutant adult males. Of the 25 males, the three oldest were included in the study because they had proven to be fertile, as was the case for one of the 6-month-old

<table>
<thead>
<tr>
<th>No. of males</th>
<th>age (months)</th>
<th>% of seminiferous tubules with spermatozoa</th>
<th>Degree of degenerative changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>14.3</td>
<td>+ to ++</td>
</tr>
<tr>
<td>10</td>
<td>3-3.5</td>
<td>15.0</td>
<td>+ to ++</td>
</tr>
<tr>
<td>5</td>
<td>4-4.5</td>
<td>16.2</td>
<td>+ to ++</td>
</tr>
<tr>
<td>5</td>
<td>5-6</td>
<td>13.6</td>
<td>+ +</td>
</tr>
<tr>
<td>3</td>
<td>9-12</td>
<td>9.2</td>
<td>– to +</td>
</tr>
</tbody>
</table>

males. Of the remaining males, one in the 3-month-old group was tested but had failed to breed. The rest of the males were prepared for histological study without having been tested for fertility. The table lists percentages of semini-
ferous tubules containing spermatozoa, and degree of intensity of degenerative changes as none (−), medium (+) and strong (++) in males of various ages. Degree of spermatogenesis ranges from 3% to 23% in mutant males, which is significantly below the range of 51–62% reported for normal fertile males of various inbred strains at the ages of 2–4 months (Pokorná, Vojtišková, Rychliková & Chutná, 1963; Vojtišková et al. 1965).

Various degenerative phenomena were conspicuous in almost all mutant males. First of all, the incidence of binucleate and giant multinucleate cells seemed to exceed that observed in normal spermatogenesis, indicating a possible increase of defects of cell division in the mutant. An additional and even more frequent difference from normal was the observation of necrotic changes in mutants. The cells had become enlarged and pale. Cell outlines had disappeared and there was much evidence of cellular fusions. Some nuclei appeared shrunken and pycnotic while others were swollen and vacuolated and had lost their capacity to be stained with basic dyes (Figs. 6, 7). The necrotic changes seemed to concern primarily the spermatids, in various stages of spermiogenesis. They were most severe in tubules with the maximum frequency of spermatids and maturing spermatozoa; besides apparently normal spermatids there were many necrotic cells with nuclei of bizarre shape, sometimes resembling huge spermatid nuclei (Fig. 6). Not infrequently necrotic late spermatids still attached to the Sertoli cells seemed to fuse with each other.

Finally, sections of the mutant epididymi showed the passing into the ductus of precursor cell stages (Fig. 8), as well as the normally expected mature spermatozoa.
Viability of $t^{AE5}/t^{AE5}$ sperm did not differ from that of control sperm. Most of the $t^{AE5}/t^{AE5}$ sperm had normal morphology although the sperm strips from $t^{AE5}/t^{AE5}$ males contained more spermatocytes and amorphous cellular debris than did those from control males. The increased frequency of cellular debris in $t^{AE5}/t^{AE5}$ sperm strips correlates well with the necrotic changes in spermatogenic cells observed histologically. Motility of sperm from $t^{AE5}/t^{AE5}$ males ranged from $+$ to $++$ while that of normal males was uniformly $++++$.

**Ovaries**

In none of the 14 females examined did the ovaries display any gross morphological deviations. Ovaries of adult females (4–12 months old) included all developmental stages of Graafian follicles and corpora lutea. Thus, in contrast to males, the poor reproductive performance of mutant females was not reflected in the morphology of the gonads.

**DISCUSSION**

Abnormalities of the lymphoid system and of spermatogenesis were shown to characterize mice homozygous for the recessive allele $t^{AE5}$ at the T-locus, which have low viability and fertility. Thymus and spleen are severely affected in both sexes, in contrast to the gonads which are abnormal in the male only. The interpretation of the abnormalities reported here must take into account the fact that the majority of $t^{AE5}$ homozygotes do not survive past weaning age. Therefore the adult animals studied represent a group selected because of their survival. It is probable that this genotype is in fact responsible for lethal defects more severe than those described here.

The association of runting, low viability, deficiencies of the lymphoid system and of reproduction in $t^{AE5}$ homozygotes is not unique but may be found in certain other genetic defects in mice. For example, reduction of growth rate, low fertility and an underdeveloped reproductive system are characteristic of ‘nude’ mice with thymus dysgenesis and a deficient immune response (Flanagan, 1966; Pantelouris & Hair, 1970; Wortis, Nehlsen & Owen, 1971; Pritchard & Micklem, 1972; Pritchard, Riddaway & Micklem, 1973; Shire & Pantelouris, 1974). Furthermore, the genetically complex NZB mouse shows structural changes in spleen and thymus in both sexes, in addition to reproductive deficiency in males (Bielschowsky, Helyer & Howie, 1959; Viklický & Poláčková, 1969; Fernandes, Yunis & Good, 1973). Finally, experimentally induced autoimmune aspermatogenesis is associated with morphological abnormalities of the lymphoid system (Vojtíšková et al. 1965). Although $t^{AE5}$ homozygotes were not tested for their immune capacity, their viability might be suggestive of a deficient immune responsiveness.

Whereas mutant ovaries appeared normal, spermatogenic activity was found to be reduced severely. The minor defects observed in mutants during
meiosis do not seem to block spermatogenesis which is able to proceed to the spermatid stage. However, though present in at least normal numbers, spermatids frequently failed to differentiate into functional spermatozoa in the mutant homozygote; they degenerated and died at various stages of spermiogenesis. In this respect, the effects of the $t^{AE2}$ allele on male reproduction appear to differ from those of previously studied $t$-alleles (Braden, 1973; Gluecksohn-Waelsch, 1972; Braden et al. 1972), but might resemble those of $t^{w2}$ (Dooher & Bennett, 1974). It is interesting that yet another $t$-allele has now been shown to have an effect on male reproduction.

The possible relationship of the pathological changes in the lymphoid system to the deficient reproductive function is unknown. There are some indications that in the nude mouse the correction of the defective immune capacity (Wortis, Nehlsen & Owen, 1971) also tended to improve reproductive function (Shire & Pantelouris, 1974).

The complex of developmental defects in $t^{AE5}$ homozygotes finds a parallel in experiments where administration of a steroid anti-androgen caused abnormalities in both spermatogenesis and the lymphoid system (Poláčková, Vílkicky & Vojtišková, 1975). The resulting phenocopies point to androgen-regulated developmental processes as possible targets of both the anti-androgen and the mutated $t$-allele. Thus, it is possible that a hormonal defect might be implicated in the genetic abnormality.

The effects of $t^{AE5}$ on thymus, spleen and testes are compatible with a previously expressed interpretation of $T$-locus effects on development and differentiation. It was suggested (Gluecksohn-Waelsch & Erickson, 1970) that the $T$-locus may control cell surface properties involved in cell-to-cell recognition, cell interactions and morphogenetic movements during embryogenesis. In $t^{AE5}$ mutants it cannot yet be decided whether such cell-surface properties involve the action of hormones, or other aspects of cell recognition and interactions.

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