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A MONOGENIC SENILITY SYNDROME SEGREGATING WITH LONGEVITY IN MICE

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SUMMARY

We have found that around 2 years of age all surviving CBA T6/T6 mice develop hyperactivity and progressive weight loss, terminating in death, which is preceded in the males by priapism, persistent penile erection. As there is no genital lesion, the priapism is presumably of neurogenic origin, providing an invaluably specific sign of development of a neurological lesion. A loss of neurons, somewhere in the brain stem, not detectable without computerised, automated microscopy, not yet applied, is at present the best explanation for the occurrence of the syndrome. In maternally-derived F2 hybrids with the NZW and C57 BL/6 strains, the syndrome occurs exactly as in the CBAs, with a frequency of 25%, indicative of mediation by a single gene or gene cluster. The syndrome also occurs in the F1 hybrids, but with a 34-week delay, suggesting a delaying effect of either a halved CBA gene dosage, or of non-CBA genes. In NZW F2 hybrids the syndrome segregates with longevity ($P < 0.001$). The phenomenon provides an animal model for study of mechanisms of ageing and their relationship to senile neuropathies, such as Alzheimer's disease.

Key words: Genetically-determined longevity; Senile neuropathy; Alzheimer's disease; CBA T6/T6 mice; Animal model

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INTRODUCTION

In the course of studies of the pathogenesis and genetics of autoimmune diseases, in man and in mice [1], we became aware of the involvement of somatic gene mutations, occurring in the immunoglobulin V (variable region) genes of B lymphocytes. Such mutations create the pathogenic forbidden (self-antigen-reactive) clones responsible for B lymphocyte-mediated autoimmune diseases, such as Graves' disease [2] and myasthenia gravis [3]. To study factors influencing the occurrence of somatic mutations in general, we measured its rate in thyroid cells of mice treated with a goitrogen (methylthiouracil). Goitrogens inhibit thyroid hormone production, causing the pituitary gland to secrete much increased amounts of its thyroid-stimulating hormone, in an unavailing effort to correct the thyroid hormone deficiency. The abnormally-stimulated thyroid cells show mutations in the form of thyroid tumours, which can be analysed, qualitatively and quantitatively. In the course of these studies, we maintained groups of mice for their whole lives. Amongst the untreated controls was a group of mice of the inbred strain, CBA T6/T6.

The study reported in this paper was initiated when one of us (J.S.S.) noticed that all the animals in three boxes of untreated CBA T6/T6 mice, 134 weeks old, had changed from being fat and lazy to being hyperactive and thin. This was followed in the male animals by development of priapism, permanent penile erection, which soon became universal [4]. In years of study of mouse populations, we had never before encountered a syndrome which occurred with such universality and uniformity. It was in striking contrast to mouse autoimmune diseases, which, because of involvement of somatic gene mutation, a random process, show wide variation in time of onset and in pathological manifestations, even in in-bred animals, all possessing the same germline genes. The universality and uniformity of the CBA T6/T6 syndrome suggests a differentiation-like process, the switching on or off of genes, like the onset of puberty. Additional interests of the syndrome are its onset in old age and its germline genetic basis, in being confined to a single inbred strain of mouse. Impressed by all this, we have made a systematic study of the phenomenon.

MATERIALS AND METHODS

Mice

CBA T6/T6 inbred strain. The CBA strain of inbred mice was developed by Strong [5]. The T6/T6 substrain [6] is widely used in research because it bears a chromosomal anomaly which can be detected by karyotypic examination, enabling its cells to be distinguished from those of other strains of mice. A translocation between chromosomes 14 and 15, translocation 6, produced a shortened chromosome 14, which is readily recognisable [6]. The breeding nucleus of our colony was obtained from Harwell in 1962 [7].

NZW inbred strain. The New Zealand white (NZW) inbred strain was developed

in Dunedin by W. H. Hall. It is healthy. Crossed with the New Zealand Black (NZB) strain, it produces offspring which develop lupus nephritis [8]. Like the NZB strain, the NZWs were derived from mice brought to New Zealand by Hall in 1930, from the colony at the Imperial Cancer Research Fund Laboratoties at Mill Hill, London.

C57 BL/6 inbred strain. This strain was developed at the Jackson Laboratories in the United States [9], the mice we used coming from a colony being maintained in Dunedin.

Diet. Mice were fed pellets, through perforated hoppers, supplemented by whole wheat. The pellets comprised a balanced rodent diet fortified with vitamins and minerals. The cereal came from barley, wheat and wheat meal, peas and maize. Animal protein came from meat and bone meal, fish meal and milk powder. A vitamin and mineral premix was included. Teeth were checked in animals losing weight to ensure they were not having difficulty with eating.

Housing. At the time when the syndrome was discovered, up to 30 mice were kept in stainless steel cages (445 × 280 × 127 mm.), the numbers decreasing as animals died. For the subsequent genetic studies, there was a maximum of 12 mice per cage. Bedding was wood shavings, changed weekly and cages were cleaned twice weekly.

Pathogens. There are no special ones of which we are aware and no obvious epidemics occurred in the course of the studies. We did experience periods when up to 20% of animals were infected with mites. An infectious origin of the senility syndrome was tested for by transferring mice between cages, with negative results.

Rearing. At weaning (3–4 weeks of age), mice were separated by sex and retained in litter lots, which were combined for mice born within a week of each other.

Recording of data. Animals within each cage were assigned a mean age (all fell within a range of 1 week) and were weighed at 4-weekly intervals. Mice were checked for death daily, when food and water were topped up and for priapism and hyperactivity in the course of cage cleaning.

Because of the original reason for which they were being kept (thyroid studies) the mice depicted in the figures were not individually numbered, this being done only after discovery of the priapism and senile weight loss and for the subsequent genetic experiments.

Measurement of hyperactivity

To establish the existence of this sign, the hole board technique [10] was used, in which individual mice are placed on a board perforated by 3.2-cm diameter circular holes and criss-crossed with lines. Over 2-min periods the number of times the mouse crossed a line or placed its head in a hole was counted. In the genetic studies, there was uniformity of observation in that the one person (W.O.L.) who was cleaning the cages also took note of the instances of hyperactivity, comparing eccentric behaviour against his familiarity with the usual background of the mouse activity. We await the application of electronic recording of the hyperactivity.

Test for thyrotoxicosis

To check whether thyrotoxicosis was involved in the weight loss and hyperactivity of the CBA mice, we gave half of them propranolol 0.04% in their drinking water for 6 days. For equivalent body weight, this dosage is 16 times greater than the highest used in man, namely 40 mg, four times a day [11].

Genetics

Breeding F1 and F2 hybrids. Female CBA T6/T6 mice were mated with males of the NZW and C57 BL/6 inbred strains, to produce F1 hybrids, which were mated together to produce F2 hybrids.

Statistical methods [12]

Measurement data. To determine the significance of differences between the means of measurements from two sources of data, Student's *t*-test was used.

Enumeration data. To determine the significance of differences between numbers in groups, the chi square test (χ^2) was used.

Regression. To determine the significance of differences between rates of loss of body weight in old age, the regression coefficient, *b*, was used.

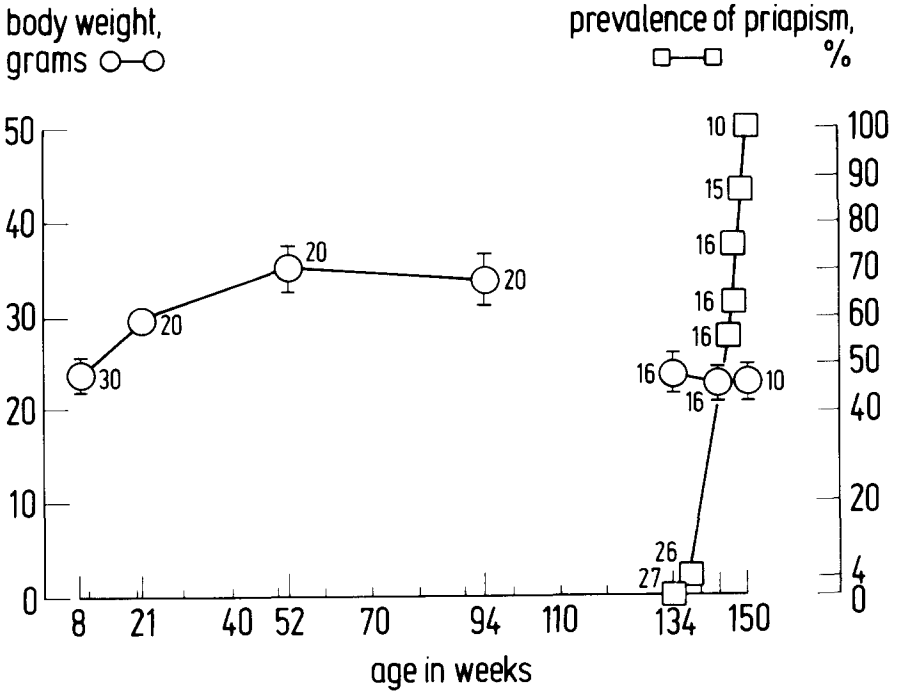

Fig. 1. The time course of body weight gain, then senile weight loss and development of priapism, in a single box of male CBA T6/T6 mice. The internal figures show the number of mice for each point. The senile phenomena being unexpected, the animals had not been individually numbered, having been set up as controls for a thyroid gland study.

RESULTS

Clinical features of the CBA T6/T6 senility syndrome

Senile weight loss. Figure 1 shows the time course of body weight changes and occurrence of priapism in a single box of male CBA T6/T6 mice. Figure 2 shows the striking universality and uniformity of magnitude (averaging 29%) of the weight loss between 94 and 134 weeks of age, in the same box of mice. A second box of 11 male animals, which had not been weighed at 94 weeks of age, at 134 weeks of age had a mean weight of 24.82 ± 1.40 g (standard deviation), closely similar to the 23.94 ± 2.02 g for the 16 males in the first box. Similar groups of female mice had mean weights 4–5 g lighter than the males, with similar variation at 8, 21 and 52 weeks of age. The females were not weighed at 94 weeks, but at 134 weeks, 14 of them had a mean weight of 24.57 ± 1.34 g, not significantly different from the males. Thus, the senile weight loss occurred in both sexes.

Priapism. Figure 3 shows a CBA T6/T6 mouse, 150 weeks old, with priapism and another, 89 weeks old, without. The abruptness of the development of priapism is shown in Fig. 1. It was first noticed in one animal, at age 138 weeks. By 150 weeks of age, all 10 surviving male mice were affected. No mice without priapism were electively killed for the histological studies.

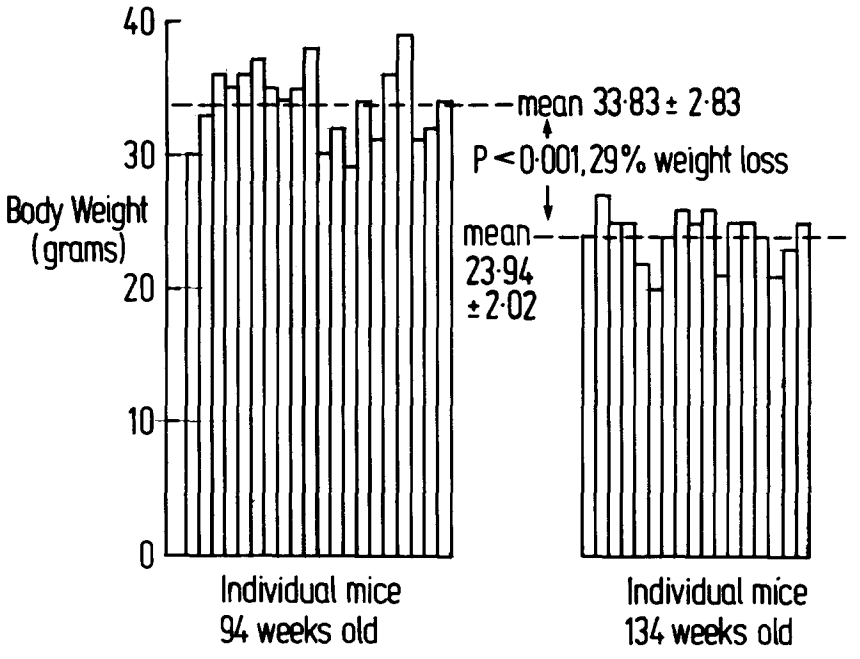

Fig. 2. The remarkable universality and uniformity of magnitude of the senile weight loss in the old CBA T6/T6 mice. Errors shown, ± 2.83 and ± 2.02, are standard deviations.

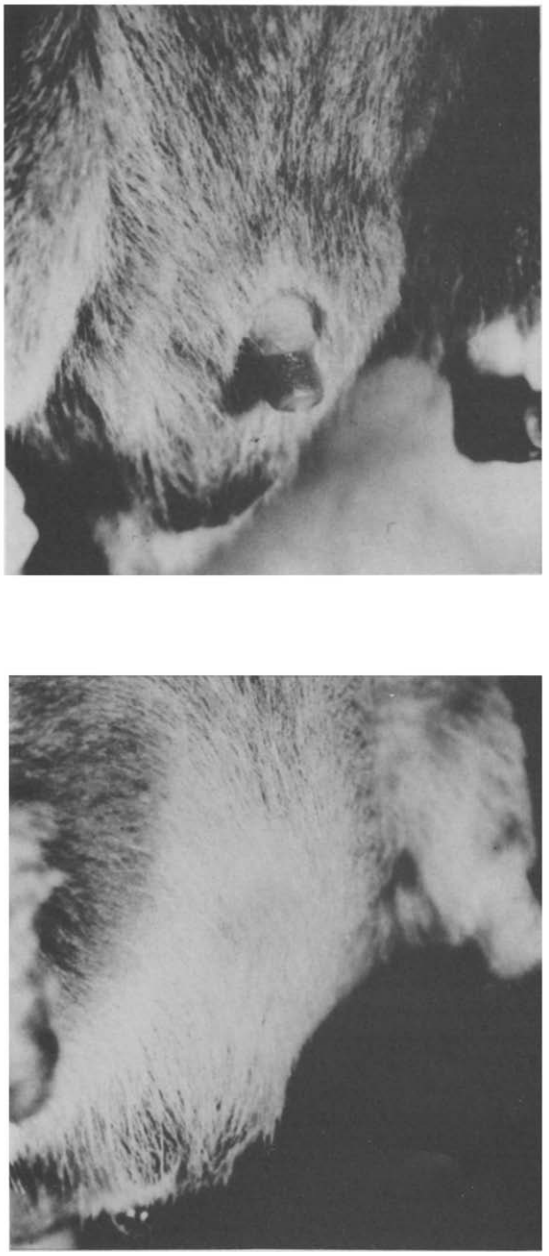

Fig. 3. A 150-week-old CBA T6/T6 mouse with priapism and an unaffected one aged 89 weeks with the flaccid penis buried in the fur.

Hyperactivity. Using the hole board technique [10], we observed 8 affected, 150-week-old male mice to have a mean of 36.0 ± 14.0 (standard deviation), line-crossing and hole-exploring actions per minute. This was significantly more ($P < 0.01$) than the 22.3 ± 5.2 actions of 4 unaffected, 89-week-old male CBA T6/T6 mice.

Ataxia. The affected mice were motionless, apart from breathing, when asleep but their movements were ataxic, with resemblance to the intention tremor seen in people with cerebellar lesions. During hole board tests of activity, the affected mice actually fell through holes in 8 of 12 trials, whereas the unaffected mice had no falls in 10 trials ($\chi^2 = 7.8$, $P < 0.005$).

Absence of thyrotoxicosis. In man, the tachycardia, tremor and weight loss of thyrotoxicosis can be corrected swiftly by administration of β-adrenergic receptor-blocking drugs, such as propranolol [11]. A trial of this had no effect on the weight or activity of the mice, excluding thyrotoxicosis.

Blood counts. In 9 affected CBA T6/T6 mice red cell counts and neutrophil counts were within the usual range for mice [13], as were the values in 3 unaffected CBA T6/T6 mice 89-weeks-old. The lymphocyte counts in both the affected and unaffected CBA T6/T6 mice were somewhat reduced at 38% and 30% of the usual values [13], respectively. There was no leukemia or leukocytosis.

Previous record of the syndrome

In 1965, CBA T6/T6 mice, the ancestors of our present colony, were being studied by other workers, in the Pathology Department, here in the Otago Medical School.

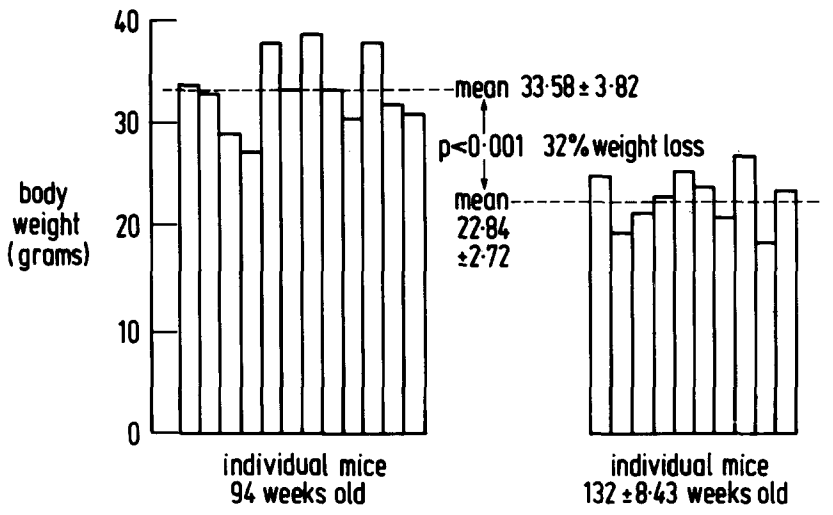

Fig. 4. Similar universality, uniform magnitude and timing of senile weight loss in CBA T6/T6 mice, in Dunedin, 21 years earlier, in 1965.

Although nothing was published, their records (courtesy of Dr. L.O. Simpson) show that the mice developed strikingly similar magnitude and uniformity of weight loss between 94 and 132 weeks of age (Fig. 4). Anxious to anticipate death, so as to secure good tissue sections for histological study, their technicians repeatedly recorded that the mice were electively killed because of 'loss of weight and poor condition'. Although not instructed to look for it, on two occasions the technicians recorded priapism. Thus, it appears that the late-onset neuropathy, which we have observed, was present in the Dunedin CBA T6/T6 colony in 1965. It has occurred again, exactly similarly, in 1988, in a third group of animals, bred by us as controls for the genetic studies. In the intervening years the colony was not studied, being maintained by the Animal Department merely to provide a source of cells identifiable by the T6 chromosomal marker.

Pathology

Priapism. The gross and microscopic anatomy of the priapism was entirely normal for this usually physiological state. In the absence of leukemia or any local anatomical abnormality, we suspect that the priapism is neurogenic, caused by a spinal reflex freed from inhibition by a higher centre, as is seen in man following transverse myelitis or injury of the spinal cord and sometimes in association with hemorrhage into the middle lobe of the cerebellum [14].

Nervous system. Light microscopy studies of the central and peripheral nervous system did not reveal any lesions. However, with the manual methods available to us, it was not possible to scrutinise the brain, or even the brain stem, in sufficient

TABLE I

PRIAPISM IN THE CBA MICE AND THE F1 AND F2 GENERATIONS OF THE CROSSES WITH THE NZW AND C57 STRAINS

Mice	*Priapism*		
	Frequency	*% Age*	*Onset age (weeks)*
CBA	14/20	70	1168 ±
(CBA × NZW)F1	3/20	15	148 ± 17[a]
(CBA × C57)F1	1/20	5	160
Both F1 groups	4/40	10	151 ± 15[b]
(CBA × NZW)F2	9/40	23	133 ± 15[c]
(CBA × C57)F2	5/17	29	124 ± 17
Both F2 groups	14/57	25	129 ± 15[d,e]

Difference from CBA mice: [a]$P < 0.001$; [b]$P < 0.001$; [c]$P < 0.005$; [d]$P < 0.025$.
Difference between F1 and F2: [e]$P < 0.025$.

detail to find a focal loss of neurons. The mouse brain stem is about 7 mm in rostral caudal length, the cross-sectional area of the medulla being about 150 square mm and that of the pons and mid-brain being about 340 square mm in both instances. The magnification needed to identify neurons adequately is 400-fold. This gives 16 microscopic fields to cover the whole cross-sectional area of the pons or mid-brain. With tissue sections 12 μm thick, about 600 serial sections are needed to cover the entire length of the brain-stem, giving a total of about 10 000 fields needing scrutiny. Hence, discovery of a loss of cells in one or more of the myriad nuclei (discrete clusters of neurons) which litter the brain stem is not possible without automated, computerised neuron counting of contiguous brain sections. This would not be difficult and would provide a neuron-count atlas. A similar atlas is needed for human neuroanatomy and neuropathology. The CBA syndrome of hyperactivity, ataxia, weight loss and priapism, seems most likely to be caused by a lesion somewhere in the brain stem, possibly affecting the reticular-activating system and causing priapism by an upper motor neuron type lesion [15–17].

Genetics

Conservation of the syndrome in filial generations. Priapism developed in 3 of the 20 NZW F1 male hybrids and in 1 of the 20 C57 ones (Table I). This is significantly less frequent than in the CBA mice ($\chi 2 = 20$, $P < 0.001$). In the F2 mice, priapism

TABLE II

CONSERVATION OF THE SENILITY SYNDROME IN THE FILIAL MICE

Male mice	*Rate of mid-life weight loss, g/week*	*Rate of senile weight loss (swl), g/week*	*Priapism onset time*	
			From swl onset, weeks	*To death, weeks*
CBA:	0.10 $n = 20$	0.53 ± 0.13 $n = 12$	26.4 $n = 14$	10.2 ± 5 $n = 14$
NZWF1:	0.03 ± 0.03 $n = 3$	0.65 ± 0.09 $n = 3$	23.7 ± 4.0 $n = 3$	6.3 ± 8.5 $n = 3$
C57F1:	0.17 $n = 1$	0.50 $n = 1$	32 $n = 1$	13 $n = 1$
All F1:	0.07 ± 0.07 $n = 4$	0.61 ± 0.02 $n = 4$	25.8 ± 5.3 $n = 4$	8.0 ± 7.7 $n = 4$
NZWF2:	0.08 ± 0.07 $n = 9$	0.46 ± 0.13 $n = 8$	26.0 ± 10.1 $n = 8$	5.3 ± 4.0 $n = 9$
C57F2:	0.05 ± 0.08 $n = 5$	0.45 ± 0.17 $n = 5$	27.8 ± 15.2 $n = 5$	5.6 ± 3.2 $n = 5$
All F2:	0.07 ± 0.07 $n = 14$	0.463 ± 0.14 $n = 13$	26.7 ± 11.7 $n = 13$	5.4 ± 3.6 $n = 14$
All filial mice:	0.07 ± 0.07 $n = 18$	0.50 ± 0.15 $n = 17$	26.5 ± 10.4 $n = 17$	6.0 ± 4.7 $n = 18$

occurred in 9 of the 40 NZW F2 males and in 5 of the 17 C57 ones, giving a combined frequency of 14 out of 57, which is 25% (Table I). In all the filial mice, priapism was preceded by substantial weight loss, averaging 30.5 ± 9.2%, and heralded death within a few weeks, just as in the CBA mice. The body weight of the CBA and filial mice reaches a plateau which is followed by a slow, midlife weight loss, as shown in Table II. In the future priapic mice this abruptly increases, about 6-fold, to give a senile weight loss of approximately 0.5 g per week, followed in about 26 weeks by priapism which heralds death in about 8 weeks. The consistency of these features in the various groups of mice is striking (Table II).

Evidence of a monogenic basis. The 25% frequency of priapism in the genetically heterogeneous F2 mice equals that to be expected from F2 re-pairing of a single recessive gene or gene cluster. Table III shows a more precise analysis, taking account of the frequency of priapism in the CBA and F1 animals. In the NZW F2 hybrids, 9 developed priapism compared to the 10 expected; in the C57 F2 hybrids 5, compared to the 3 expected, giving a combined number of 14 compared to the 13 expected. Hence, the frequencies suggest that the priapism is caused by a single gene or gene cluster, with a reduced expression in the heterozygous state.

Segregation of the syndrome with longevity. Table IV shows that priapism and senile weight loss segregate with longevity in (CBA × NZW)F2 hybrids. This means that genes influencing life span are involved in the syndrome.

TABLE III

EVIDENCE THAT THE PRIAPISM IS MONOGENIC. ANALYSIS OF THE F2 FREQUENCES IN THE LIGHT OF THOSE OF THE CBA AND F1 MICE

Mice	*Total*	*Expected genetic composition*	*Number with priapism*	
			Expected	*Observed*
1. (CBA × NZW)F2	40	10 CBA homozygotes × 70% =	7	
		20 heterozygotes × 15% =	3	
		10 NZW homozygotes × 0%[a] =	0	
		Total	10	9
2. (CBA × C57)F2	17	4.25 CBA homozygotes × 70% =	3.0	
		8.5 heterozygotes × 5% =	0.4	
		4.25 C57 homozygotes × 0%[a] =	0	
		Total	3	5
3. Combined F2s	57	14.25 CBA homozygotes × 70% =	10.0	
		28.5 heterozygotes × 10% =	2.9	
		14.25 non-CBA homos × 0%[a] =	0	
		Total	13	14

[a]Presumed.

TABLE IV

SEGREGATION OF PRIAPISM WITH SENILE WEIGHT LOSS AND LONGEVITY IN (CBA × NZW) F2 MALE MICE

Mice	*Number of mice*	*Senile weight loss, %**	*Life span in weeks*
Priapic	9	30.6 ± 5.9	137.4 ± 15.3
Non-priapic	31	13.4 ± 7.8	106.3 ± 22.3
	Difference:	17.2 ± 2.8	31.3 ± 8.0
		$P < 0.001$	$P < 0.001$

*Maximum weight minus weight 4 weeks ante-mortem.

Delayed onset in the F1 hybrids. The mean age of onset of priapism in the F1 mice was 35 weeks later than in the CBA mice (Table I), a highly significant difference, $P < 0.001$. In the F2 mice the mean onset age was intermediate. On the assumption of a monogenic basis, the 14 F2 priapic mice are calculated to be comprised most probably of 10.9 CBA homozygotes and 3.1 heterozygotes (70% expression in 14.25 homozygotes = 9.98, compared with 10% expression in 28.5 heterozygotes = 2.85, giving a predicted homozygote/heterozygote ratio of 3.5/1.0 in the 14 priapic mice, therefore the number of heterozygotes = 141/(3.5 + 1.0) = 3.1). Hence, the predicted mean onset time for priapism in the F2 mice is (10.9 × 116) + (3.1 × 151) all divided by 14. This comes to 124 weeks, close to the observed time of 129 weeks. Thus, the onset times are also compatible with causation by a single gene or gene cluster.

TABLE V

LONGEVITY-RELATED SENILE WEIGHT LOSS IN THE CBA MICE AND IN F1 HYRBRIDS WITH C57 MICE

Mice	*Number of mice*	*Regression of senile weight[a] on life span*		
		b[b]	*P[c]*	*P[d]*
a. Males				
CBA	20	−0.22 ± 0.05	<0.001	
(CBA × C57)F1	20	−0.20 ± 0.04	<0.001	N.S.[e]
b. Females				
CBA	20	−0.17 ± 0.02	<0.001	
(CBA × C57)F1	20	−0.20 ± 0.08	<0.001	N.S.[e]

[a]4 weeks ante-mortem.
[b]Regression coefficient, g lost per week of life span.
[c]Significance of regression.
[d]Difference from CBAs.
[e]Not significant.

TABLE VI

ABSENCE OF LONGEVITY-RELATED SENILE WEIGHT LOSS IN CBAF1 HYBRIDS WITH NZW MICE

Mice	*Number of mice*	*Regression of senile weight*[a] *on life span*		
		b[b]	P[c]	p[d]
a. Males				
CBA	20	−0.22 ± 0.05	<0.001	
(CBA × NZW)F1	20	−0.11 ± 0.08	N.S.[e]	<0.001
b. Females				
CBA	20	−0.17 ± 0.02	<0.001	
(CBA × NZW)F2	20	−0.05 ± 0.06	N.S.[e]	<0.001

[a]Four weeks ante-mortem.
[b]Regression coefficient, g lost per week of life span.
[c]Significance of regression.
[d]Difference from CBAs.
[e]Not significant.

As the interval between onset of senile weight loss and onset of priapism is the same for the CBA and F1 mice (26 weeks, Table II), the onset of the pre-priapic senile weight loss is also 35 weeks later in the heterozygous mice. This later onset of the senility syndrome in the F1 mice may be the cause of its reduced frequency. The mean age for onset of priapism in the CBA mice is 4 weeks short of the mean age of death, but in the F1 mice it is 18 weeks beyond it.

Differences between the NZW and C57 hybrids. Sporadic weight loss is common in groups of old mice. However, when the effect of terminal disease events is reduced by measuring only up to 4 weeks ante-mortem, the CBA and (CBA × C57)F1 mice show a highly significant, longevity-related senile weight loss (Table V). In contrast, the (CBA × NZW)F1 mice do not (Table VI). This suggests that the NZW and C57 strains have different genes, possibly allellic, affecting senile weight loss. There is no difference between the sexes (Tables V and VI).

TABLE VII

LACK OF CONFINEMENT OF SENILE WEIGHT LOSS AND LONGEVITY TO PRIAPISM IN (CBA × C57) F2 MALE MICE

Mice	*Number of mice*	*Senile weight loss, %*	*Life span in weeks*
Priapic	5	32.8 ± 13.7	130.2 ± 20.8
Non-priapic	12	25.9 ± 17.0	125.4 ± 19.9
	Difference:	6.9 ± 8.7	4.8 ± 10.7
		N.S.	N.S.

TABLE VIII

SINGLE GENE INTERPRETATION OF THE OBSERVED PHENOTYPES IN THE PARENTAL AND FILIAL MICE

Mice	*Genotypes*	*Phenotypes*		
		Priapism	*Senile weight loss*	*Longevity*
CBA:	Senility syndrome gene (SS)	++++	++++	++++
NZW:	Wild type gene (ss)	–[a]	–[a]	–[a]
C57:	C57 type gene (CC)	–	?	++++[b]
(CBA x NZW)F1	Ss	+[c]	+[c,d]	++++[d]
F2	SS	++++	++++[d]	++++[d]
	Ss	+[c]	+[c,d,e]	++++[d]
	ss	–	–	–
(CBA × C57) F1	SC	+[c]	++++[e]	++++[d]
F2	SS	++++	++++[d]	++++[d]
	SC	+[c]	++++[e]	++++[e]
	CC	–	++++[g]	++++[e,h]

[a]Presumption, untested.
[b]C57 BL/6 mice are long-lived [9].
[c]Of later onset and less frequent.
[d]Priapism-associated.
[e]Additional to priapism-associated.
[f]Less than in the C57 F2 mice, $P < 0.005$, Tables IV and VII.
[g]Bottom C57 F2 quartile is greater than the NZW one, $P < 0.001$.
[h]Bottom C57 F2 quartile is greater than the NZW one, $P < 0.01$.

In the F2 generations the strain differences continue. Table VII shows that, in contrast to their NZW F2 counterparts, the C57 F2 males do not show segregation of priapism with senile weight loss and longevity. The priapic C57 F2 males do not differ from the NZW ones, but the non-priapic C57 F2 males show significantly more senile weight loss and longevity (Table VII).

In the C57 F2 mice there was a significant deficit of males. Only 17 were weaned, compared to 38 females ($\chi^2 = 7.27$, $P < 0.01$). In the NZW F2 mice there was no sex imbalance, 40 males and 40 females being produced without difficulty. The C57 F2 male deficit seems to be random relative to the priapism, which had similar frequency and onset times in F2 males of both crosses.

Single gene interpretation. Table VIII shows that the observations can be accounted for by a single CBA gene (or gene cluster) with different alleles in the NZW and C57 BL/6 strains. The latter strain, with a 50% survival time of 129 weeks, resembles the CBA T6/T6 strain in being unusually long-lived [9]. In the F2 mice, the top three quartiles for life span do not differ significantly between the NZW and C57 hybrids, at 132 and 134 weeks, respectively. However, for mice living more than 1 year, the bottom quartiles show shorter life span in the NZW hybrids, at 78 weeks,

compared with 92 weeks for the C57 hybrids ($P < 0.01$). This may reflect homozygosity for a wild-type death clock gene in the NZW mice, as discussed below.

The greater senile weight loss in the C57 non-priapic F2 males over the NZW ones (Tables IV and VII) is consistent with 75% F2 expression of a CBA gene for senile weight loss, which is not inhibited by the C57 allele, but is by the NZW allele, priapism being inhibited by both the NZW and C57 alleles. In support of this, senile weight loss of 25% or more occurred in a greater number of C57 than NZW male and female F2 mice ($\chi^2 = 5.65$, $P < 0.025$), the difference increasing when only the lower three quartiles are considered, with 6 out of 60 NZW and 16 out of 41 C57 F2 hybrids showing the heavy senile weight loss ($\chi^2 = 10.4$, $P < 0.005$). Finally, the

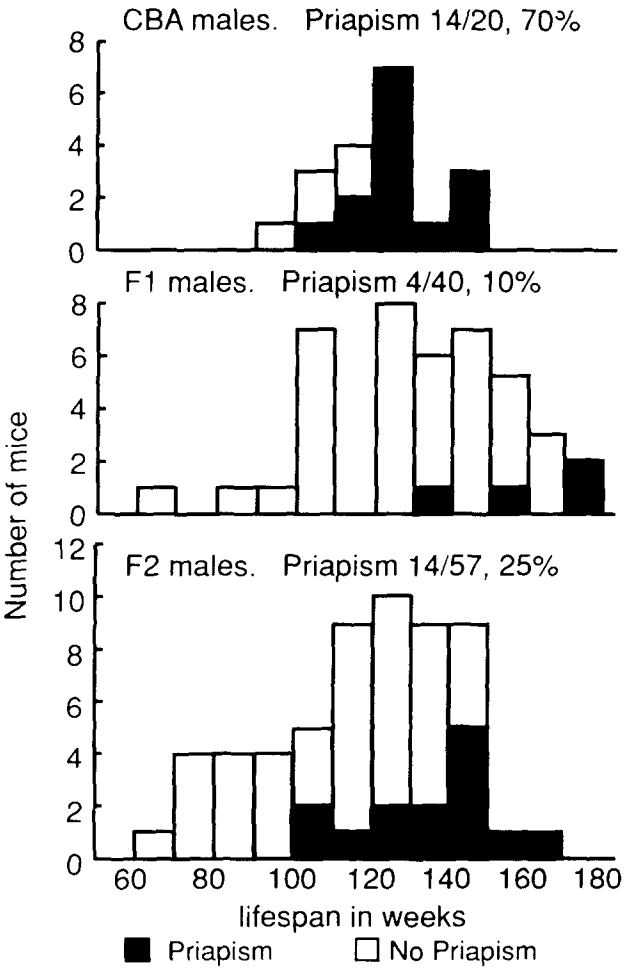

Fig. 5. Association of priapism with longevity in CBA T6/T6 mice and in both the F1 and F2 hybrids with the NZW and C57 BL/6 inbred strains.

bottom quartile of C57 F2 senile weight loss, at 7.1 ± 3.5%, is greater than that of the NZW F2 mice, at 2.6 ± 2.6%, $P < 0.001$.

The question arises as to whether senile weight loss occurs in the C57 BL/6 strain, similarly to in the CBA T6/T6 strain? Both the NZW and C57 BL/6 strains need to be studied for occurrence of features of the CBA senility syndrome.

Hyperactivity. This was observed in all the priapic males of the filial generations and in 10 of the 46 NZW F2 and C57 F2 females surviving more than 100 weeks. This is a frequency in the F2 females of 22%, again approximating 25% and so suggesting a monogenic basis, similarly to the frequency of priapism in the F2 males. The hyperactive F2 female mice had a mean senile weight loss of 38.6 ± 11.2% (% weight lost from maximum to 4 weeks antemortem) compared with 20.9 ± 12.8% in their non-hyperactive siblings, a highly significant difference, $P < 0.001$. Thus, there is a close association between hyperactivity, senile weight loss and priapism. It is possible that the hyperactiviy is the cause of the senile weight loss. An electronic method of measuring the hyperactivity is needed.

Association with longevity. For the NZW F2 male mice, the segregation of priapism and senile weight loss with longevity ($P < 0.001$) is shown in Table IV. Figure 5 shows the occurrence of priapism in relationship to life span for all the CBA, F1 and F2 males. In all three generations, the longest lived mice developed priapism.

Chromosomal location. The absence of difference between the sexes rules out the X and Y chromosomes as the site of the genes involved.

The CBA and NZW strains differ in three coat colour genes, the CBAs having full colour (C), black (B) and intense (P), in contrast to albino (cc), brown (bb) and pink-eyed dilute (pp) in the NZWs [18,19]. One of the priapic mice in the F2 generation and 3 females with characteristic senile weight loss, were albino, excluding chromosome 7 as carrying the CBA genes involved. None of the F2 priapic males was brown (bb), but 2 F2 females with characteristic senile weight loss were, suggesting that chromosome 4 does not carry the senility syndrome gene(s).

The C57 BL/6 strain differs from the CBA in having non-agouti (aa) instead of agouti (A) on chromosome 2. One of the 5 priapic (CBA × C57)F2 mice was non-agouti, so, unless there had been cross-over, chromosome 2 is also excluded.

Preliminary cytogenetic studies, courtesy of Dr. R.M. Gardner, indicate that the shortened chromosome 14 of the CBA T6/T6 mice [9] does not carry the gene(s) causing priapism. Hence, CBA chromosomes 2, 4, 7, 14 and X and Y seem to be excluded, leaving 15 candidate chromosomes. Chromosome 17 is of special interest, as it carries not only the major histocompatibility antigen complex (MHC) genes influencing the clonal specificities of the lymphocyte repertoire and, hence, susceptibility to virus infection and to autoimmunity, but also homozygous lethal genes [20], which are discussed below. Chromosome 16 is another of special interest in carrying mouse analogues of human genes related to Down's syndrome and familial Alzheimer's disease [21].

DISCUSSION

In familial Alzheimer's disease there, is a genetically-determined, late-onset loss of neurons in the cerebral cortex, with devastating impairment of memory and cognition [22]. The etiology is unknown, there being at least six conceptual models [23]. The CBA T6/T6 mouse senility syndrome is similar to familial Alzheimer's disease in being a genetically-determined, late-onset neuropathy, with the difference that its apparent loss of neurons is in the brain stem rather than the cortex.

Down's syndrome, caused by trisomy 21, the occurrence of three copies of the 21st chromosome, is one of the commonest causes of mental retardation [24]. Accordingly, this chromosome has been extensively characterised by cytogenetic and molecular techniques, including the development of physical and genetic maps [24]. Karyotypic analyses of cases of partial trisomy 21 have indicated that only a defective band 22 of the q arm of chromosome 21 is required for full manifestations of Down's syndrome [21]. This band contains a few hundred genes, no individual locus having been established as causative. Functionally-interesting genes are in the 21q22 band [21]. These include a proto-oncogene, genes for receptors for interferons (which protect against virus infection by inhibiting protein synthesis) and a gene for superoxide dismutase (SOD 1), which reduces somatic mutation rates by scavenging the highly reactive and destructive free radicles formed by the interaction of short wavelength electromagnetic radiation with water. Furthermore, familial Alzheimer's disease has been found to involve genes in the proximal half of the q arm of chromosome 21, a region which also contains the structural gene for the protein forming the amyloid plaques characteristic of Alzheimer's disease and Down's syndrome [21,25].

Mouse trisomy 16 has been proposed as an animal model of Down's syndrome, because use of human DNA probes has shown this chromosome to contain several homologues of loci in the q22 band of human chromosome 21 [21]. Unfortunately for research, such mice, made artificially by chromosome transfer, are not viable [21]. Furthermore, DNA markers from the Alzheimer's disease and Down's syndrome regions of chromosome 21 map to mouse chromosome 17 as well as to chromosome 16, showing that mouse trisomy 16 is only a partial model of Down's syndrome [21]. These limitations enhance the value of the CBA T6/T6 mouse senile neuropathy as a research tool for elucidation of human neurodegenerative mechanisms. The value of animal models of disease is well illustrated by the New Zealand Black mouse, studies on which led to the H gene theory, which has made the previously vexed genetics of the autoimmune diseases simple [1].

Conceptual models for etiology of the CBA T6/T6 senility syndrome

Mutant death clock gene. In populations of wild mice, the T and t series of genes, so designated for the tail abnormalities they cause, occur with what Klein describes as 'astonishing' frequency, being found in about a quarter of all the mice tested [20]. This high frequency indicates associated reproductive advantage. We suggest that

TABLE IX

VIRAL-MHC INTERACTION AS AN INTERPRETATION OF THE OBSERVED PHENOTYPES

MHC genotypes	*Putative CBA virus*	*Putative C57 virus*	*Senile weight loss*	*Priapism*	*Life span*
(CBA × NZW) F2 males					
25% CBA:CBA	+	–	+	+	CBA-length (longish)
50% CBA:NZW	+	–	Later	Later	Extra-long
25% NZW:NZW	?	+	Never	Never	NZW-length (shortish)
(CBA × C57)F2 males					
25% CBA:CBA	+	–	+	+	CBA-length (longish)
50% CBA:C57	+		Later	Later	Extra-long
		+	Earlier		
25% C57:C57	?	+	Earlier	Never	C57-length (longish)

Major histocompatibility gene complex, MHC. The putative CBA virus must be vertically transmitted (mother to fetus) or be an MHC-linked provirus (DNA copy of an RNA virus) to account for the monogenic inheritance. The genetic effects in this model are ascribed to MHC dictation of the immune repertoire, this influencing viral activation time consequent on senile loss of immunological clones. In the mice with NZW:NZW or C57:C57 MHCs, the CBA virus will be present if it is vertically transmitted, but not if it is a provirus linked to the CBA MHC. The earlier senile weight loss occurring in the C57 F1 hybrids is depicted as being due to an additional late-onset virus, in C57 mice, which affects both the heterozygous and homozygous hybrids.

this is from the lethality of these genes in the homozygous state, one advantage of which is the maximalisation of MHC heterozygosity, giving improved defense against infectious and autoimmune diseases [26]. Similar lethality genes becoming active in senility, may have evolved to benefit populations in the face of famine. The longevity of the CBA T6/T6 and C57 BL/6 strains may be a consequence of defective senile lethality genes. The senile weight loss and priapism might result from neuropathy mediated by viral or autoimune activity consequential on senile decline of immune function. Alternatively, a mutant death clock gene might cause the senile neuropathy directly by slowly killing the wrong neurons.

Autoimmunity. By definition, autoimmunity is the reaction of a lymphocyte clone with a host antigen instead of a microbial or other foreign one. The CBA senile neuropathy could be caused by such forbidden clones, possibly arising through loss of a protective anti-idiotypic (anti-antigen receptor) clone [1]. Again the genes involved could be those of the MHC [1]. The monogenic regularity of the CBA genetics is unprecedented for known, non-senile, autoimmune diseases, but the random element in autoimmunity [1] could explain the differences between the NZW and C57 hybrids.

Activation of a latent virus. Certain viruses can persist in their host without giving rise to the usual signs of infection [27]. Such viruses can cause or trigger chronic

neurological degenerative disease [28–30]. In the CBA mice, senile decline of immune function, particularly thymic, could permit activation of a latent virus infection. Kaplan [31] and we ourselves [32] have observed activation of latent oncogenic viruses by depression of the immunity system. Kaposi sarcoma in acquired immune deficiency syndrome (AIDS) illustrates the same phenomenon [33]. The CBA neurons could be killed directly by the virus or indirectly by clones of anti-viral cytotoxic T cells. The genes involved could be the immune specificity genes of the major histocompatibility antigen gene complex (MHC) [20,26,34–37]. Table IX shows how the observed NZW F2 and C57 F2 phenotypes can be explained by the influence of the MHC genes from the NZW and C57 BL/6 strains, together with the effect of a putative second virus, latent in the C57 mice.

CONCLUSION

The CBA T6/T6 senility syndrome provides an animal model for advancing understanding of mechanisms governing life span and their relationship to genetically-determined neuropathies, including familial Alzheimer's disease and Down's syndrome. The next objectives are clearly to find the lesion and to find its causative gene(s).

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