Degenerative arthritis in mice

Study of age and sex frequency in various strains with a genetic study of NZB/B1, NZY/B1, and hybrid mice

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SUMMARY Of a number of strains of mice studied for evidence of autoimmunity, NZY/B1 mice showed the highest frequency of degenerative arthritis of the knee, reaching a maximum of 68% at 18 months. 50% of PN mice were affected at 24 months. Both breeds differed from previously described strains in not showing a sex difference. The NZB/B1 mice were rarely affected and hybrids to the NZY and the backcross to the NZB showed a recessive pattern of inheritance for knee arthritis and an additive pattern for carpal arthritis. Polygenic inheritance was postulated as at least three genes would be necessary to explain the findings. Joint disease was not related to red cell and nuclear antibodies, glomerulitis, or arteritis.

The NZY/B1 mice used in this study were originally bred from the same colony of outbred mice as the NZB mice which develop autoimmune haemolytic anaemia (Bielschowsky *et al.*, 1959). Helyer and Howie (1963) described a lupus erythematosus-like disease in NZB $\times$ NZY F1 hybrids with LE cells and severe glomerulitis with focal fibrinoid changes in the capillary loops. 60% of these hybrids were affected by a severe fibrinoid arteritis identical to that found in PN mice, and similar renal changes and antinuclear reactions were found in the NZB and to a lesser degree in the NZY mice (Wigley *et al.*, 1970). The NZY mice showed a higher incidence of knee joint arthritis than NZB mice (Wigley and Highton, 1965). Joint disease in the two strains and their reciprocal F1 crosses and the backcross to the NZB is described and compared with the frequency in the other strains studied, including the PN mice described by Wigley *et al.* (1975) and shown to be unrelated to the autoimmune phenomena.

Methods

Breeding pairs of the NZY/B1 and NZB/B1 mice were provided by Dr M. Bielschowsky, the NZW/B1 by Professor J. B. Howie of Otago University, and the C57/MAC black, 101/MAC, CHI/MAC, and CBA/FaMAC by Professor R. Munford of Massey University. The PN mice were as described by

Wigley *et al.* (1970). Sagittal sections of the right knee joint were cut in mice killed during a study of the genetics of autoimmune disease in NZB mice and hybrids and in other strains. The sections were routinely stained with haematoxylin and eosin after decalcification. All other procedures were as described by Wigley *et al.* (1970). All material was read under code number.

Intraobserver variation was studied by comparing duplicate readings of 33 knee and 29 carpal preparations from NZB and NZY mice in part II of the study using the following grades: 1 = no change, 2 = doubtful change, 3 = loss of staining of cartilage cell nuclei and/or variation of cell size, 4 = fragmentation of the cartilage surface and/or formation of new cartilage at the joint margins, 5 = loss of cartilage exposing the calcified layer, 6 = more advanced changes. For the knee joint, $r = 0.9$, $P < 0.001$, $n = 33$; and for the carpus, $r = 0.8$, $P < 0.001$, $n = 29$. Grades 1 and 2 were classed as negative in the general analysis and grades 3 to 6 as positive. For the knee joints one reading was changed from positive to negative and one from negative to positive. For the carpus one reading was changed from positive to negative and two from negative to positive. Thus 92% would be unchanged at the second reading.

Results

PART I

All adequate knee joint sections from the strains

studied are listed in Table 1. 28% of the C57 black mice showed the degenerative changes described by Silberberg and Silberberg (1941) with the expected male sex dominance, 8 of the 9 affected being males. C57, CBA, and possibly the 101 mice showed a higher frequency in males than females (Table 1), but NZY, CHI, NZW, and PN mice showed no sex difference.

Table 1 *Proportion of mice in part I of the study with degenerative arthritis of the knee by breed and age. Allowing for increased frequency with age, only the C57/MAC and 101/MAC mice show a clear sex difference with a higher frequency in males*

Breed	No. examined	% affected	Mean age (m)	SD age
NZY/BI	113	43	11.2	4.1
B × Y	53	6	15.9	5.4
F1				
B × (BY)	42	7	13.5	4.4
BC				
NZB/BI	92	3	12.9	3.7
NZW/BI	33	12	14.5	6.4
CHI/MAC	24	29	16.3	4.1
C57/MAC	32	28	15.3	4.2
CBA/MAC	50	14	11.4	3.7
101/MAC	23	39	14.6	4.9
PN (outbred)	412	14.6	14.6	8.2

The majority of sagittal knee joint sections from NZY mice showed localized loss of nuclear staining of the cartilage apparently followed in some mice by fibrillation of the surface of the articular cartilage (Fig. 1a) with an increase in cellularity and hypertrophy of cartilage at the joint margins. The superficial layer of the articular cartilage had separated in half the mice (Fig. 1b). The deeper calcified cartilage was eroded with destruction of bone and fibrosis in the adjacent marrow spaces in one-sixth of the NZY mice. The patellofemoral articulation was rarely affected. In a few mice destructive changes, bone absorption, and the formation of granulation tissue occurred, but cellular infiltration, if any, was of minor degree. The synovium was normal and pannus formation was not seen. The highest frequency of arthritis was recorded in NZY mice (Table 1). Fig. 2 shows the rising frequency with age for both sexes. There was no significant sex difference and if arthritis of more than minimal degree only was considered, the frequency was similar at 8% for males and 10% for females.

The lowest frequency was recorded in the NZB mice, only 3 of 61 males and none of 46 females being affected. The affected males were 12, 16, and 23 months old. A small sample of NZW mice showed a low frequency of arthritis with no clear sex difference. 414 closed-colony bred PN mice studied showed a rising frequency of arthritis with age

Fig. 1 *Sagittal sections of knee joints of NZY/BI mice. (a) Grade 4 changes showing loss of staining of cell nuclei and fragmentation of the articular surface of the cartilage of the tibia. H & E × 55. (b) Grade 5 change showing separation of the superficial articular cartilage exposing the calcified layer on the tibia. H & E × 30.*

(Fig. 3), reaching 50% at 24 months. None of the strains studied was entirely free of arthritis and no qualitative difference in the arthritis was noted between strains. Macroscopical joint abnormality was infrequent and was only noted in the tarsal joints. Sections of these showed aseptic necrosis of bone and some showed destruction of the tarsus and formation of granulation tissue making distinction from the effects of trauma and infection difficult, so that this change was not scored for analysis. No significant associations were shown between anti-nuclear antibodies, Coombs's tests, glomerulitis or arteritis, and histological arthritis.

Fig. 2 Percentage of NZY/BI mice affected by degenerative arthritis of the knee in relation to age in part I of the study. The dotted lines indicate the mean ages and percentages for the male (M) and female (F) mice in part II of the study. The bars indicate the 95% confidence limits (binomial).

PART II

To study the genetics of the degenerative arthritis a subsample of the NZY mice was re-examined with samples of B $\times$ F1 hybrids, the backcross to the NZB mice (B/C), and pureline NZB mice of similar sex and age at death. Sections of the carpus were made in all and adequate sections of the elbow joint in 94 of the 117 mice. Table 2 shows the age and sex distribution to be comparable.

Carpal joint changes were similar to those in the knee joints. Localized loss of staining of cell nuclei was the earliest change seen in the articular cartilage, which subsequently became irregular and ragged,

staining deeply with eosin at the free edges (Fig. 4). Overgrowth of cartilage with large deeply staining cells was noted at the joint margins with some cartilaginous metaplasia of the synovial membrane. Three-quarters of the NZY mice showed changes of at least the severity illustrated in Fig. 4a & 4b. Cellular infiltration was uncommon and of minor degree, but in some instances there was round cell infiltration and cartilage loss and in the most gross examples joint destruction with collagen formation. The changes in the elbows were similar in type, but were less marked, rarely reaching grade 5.

Fig. 3 Percentage of PN mice with degenerative arthritis of the knee with 95% confidence limits showing an increase with age.

Table 2 Age distribution of mice included in part II of the study. Mean ages for females are lower than for males, but the samples are comparable for each breed when the sexes are combined.

Breed	Sex	Total	Mean age (m)	SD age
NZY	M	19	15.0	4.7
	F	16	11.6	5.5
B $\times$ Y	M	12	16.8	4.0
	F	19	11.9	4.8
B $\times$ (BY)	M	12	16.0	4.5
	F	9	12.3	5.5
NZB	M	16	15.0	2.9
	F	13	11.8	3.8

Fig. 4 Degenerative changes in the carpal joints of NZY/Bl mice. (a) Grade 6 change showing loss of cartilage (arrow heads) and increased eosinophilia and fibrillation at the margins of the articular cartilage. H & E $\times$ 55. (b) Grade 6 change showing areas of loss of staining of cartilage cell nuclei (arrows) and ulceration of cartilage down to underlying bone (arrow heads). H & E $\times$ 60.

Knee joint arthritis was clearly more common in NZY than in the other breeds (Fig. 5). For the carpal joints the difference was much less, but between the NZY and NZB strains was significant ($\chi^2 = 7.08$; $P < 0.01$, > 0.005). Though males were marginally more frequently affected than females in the sub-groups, this difference was accounted for by the higher age of the males. Fig. 2 shows that both males and females of part II (dotted lines) conform to expectation from the whole sample in part I.

Discussion

The knee arthritis observed in this study did not differ from that described in C57/Bl mice by

Fig. 5 Percentage with 95% limits of the mice in part II of the study by breed and joints affected. Any joint indicates arthritis in either knee, carpus, or elbow joints. NZY = NZY/Bl; B $\times$ Y = NZY/Bl by NZB/Bl F1 hybrids; B/BY = the backcross of the B $\times$ Y hybrid to the NZB/Bl mice; NZB = NZB/Bl.

Silberberg and Silberberg (1941). Though C57/MAC mice used in this study were separated by many generations, the sex difference was of a similar degree. The Silberbergs (1971) have shown that castration has a protective effect in males and that the arthritis is increased when testosterone is given to castrated males and the converse holds for females, suggesting that a genetic tendency to arthritis is modified by sex hormones. The same authors (1965) consider that progesterone promotes arthritis so the problem is complex. Silverstein (1961) reported a higher frequency in males of all strains studied. An apparent sex difference in the NZY and hybrid mice in part II was attributable to the lower age of females in all groups, but no true sex difference has been shown for NZY or PN mice.

Sokoloff *et al.* (1962) found a recessive genetic pattern for knee arthritis in STR/1N hybrids using macerated skeletons and postulated polygenic inheritance to account for the wide variation in frequency in different strains. This macroscopical technique would not disclose the carpal changes shown in this study. Silverstein (1961) noted ulceration of cartilage and eburnation in guinea pig carpal joints with relatively little osteophyte formation, but carpal changes have not been previously described in mice.

At least three genes must be postulated to explain the observations. These may act directly, for instance by determining anatomical variations or

differences in the proteoglycan composition of the joint cartilage, or indirectly by determining patterns of hormone production or physical activity. Zipkin *et al.* (1967), considering the possibility that increased sclerosis of bone might affect the frequency of arthritis, were unable to alter the severity or incidence in STR/1N or A/LN mice with fluoride. Sokoloff's (1959) radiographic finding that the arthritis is asymmetrical, suggests that local environmental factors determine which joints will fail given the genetic predisposition.

Difficulty in consistently cutting sections through the centre of the femoral condyle where the greatest forces are applied and the fact that only one condyle is visible on a sagittal section shows that frontal sections are preferable for further study (Walton, 1974). Using scanning electron microscopy in STR/ORT mice which are derived from the STR/1N strain, Walton (1974) has shown that the medial condyle of the tibia is the area first affected and that in that strain medial subluxation of the patella may be aetiologically important. This was not observed in the NZY mice or their hybrids on review of the radiographs.

The steady rise in age in the two strains studied in sufficient numbers, NZY and PN, drops only in the last age category suggesting that those with no arthritis at 18 and 24 months respectively have a greater chance of survival.

The tarsal lesions differ from the idiopathic necrosis of bone described by Sokoloff and Habermann (1958) in affecting the tarsus only, but were identical to those described by Walton (1974). Radiographs in this study suggest that this lesion affects NZY mice more than the other strains. Though the changes may be similar to the destructive changes seen in the carpus of a few mice, histologically the changes are gross and are possibly not relevant to the changes in the other joints. Radiographs of the carcasses detected only the severe grades of arthritis in knee joints. Radiographs showed no arthritis in the other large joints, spine, or tail.

In conclusion, the greater susceptibility of males than females to degenerative arthritis in the previously described strains had led to concentration on the endocrine effects on this model of human disease. Two strains are described in this paper with no sex difference, suggesting that other genetic and environmental modifying factors should be sought. All the strains studied showed a progressive increase

with age. The differing frequencies in inbred strains and the different genetic patterns for the carpus and the knee joint and the effect of sex indicate that at least three genes are involved in determining the occurrence of arthritis in mice.

This work was conducted during tenure of research grants from the USPHS (AM 06650-03); the Bank of New Zealand (R.M.); the Arthritis and Rheumatism Council, London; the Palmerston North Medical Research Foundation; and the New Zealand Medical Research Council.

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