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Immunology

Life-span, T-cell responses, and incidence of lymphomas in congenic mice

(survival/lymph cell cancers/aging/immunodeficiency)

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ABSTRACT **Survival, T-cell functions, and postmortem histopathology were studied in *H-2* congenic strains of mice bearing *H-2^b*, *H-2^k*, and *H-2^d* haplotypes. Males lived longer than females in all homozygous and heterozygous combinations except for *H-2^d* homozygotes, which showed no differences between males and females. Association of heterozygosity with longer survival was observed only with *H-2^b/H-2^b* and *H-2^b/H-2^d* mice. Analysis using classification and regression trees (CART) showed that both males and females of *H-2^b* homozygous and *H-2^k/H-2^b* mice had the shortest life-span of the strains studied. In histopathological analyses, lymphomas were noted to be more frequent in females, while hemangiosarcomas and hepatomas were more frequent in males. Lymphomas appeared earlier than hepatomas or hemangiosarcomas. The incidence of lymphomas was associated with the *H-2* haplotype—e.g., *H-2^b* homozygous mice had more lymphomas than did mice of the *H-2^d* haplotype. More vigorous T-cell function was maintained with age (27 months) in *H-2^d*, *H-2^b/H-2^d*, and *H-2^d/H-2^k* mice as compared with *H-2^b*, *H-2^k*, and *H-2^b/H-2^k* mice, which showed a decline of T-cell responses with age.**

Life-span of a species is controlled by genetic factors that determine cell development and involution, environment, and interactions between the two. That mice of different strains showed differences in life-span suggested that genetic markers might be identified to explain these differences. Studies of congenic strains that differ only in a limited chromosomal region have elucidated genes controlling life-span. That major histocompatibility complex (MHC) genes were limited to life-span is provocative because MHC genes regulate immune functions, which may decline with age (1, 2). Difference in *H-2* haplotypes are associated both with differences in life-span and T-cell responses during life (3, 4). The role played by *H-2* alleles in determining life-span may be influenced by environmental factors. In two models, backcross and congenics, the *H-2^d* haplotype was associated with a shorter life-span than was the *H-2^b* haplotype, whereas in an intercross model *H-2^d* conferred survival advantage over *H-2^b*. However, congenic or backcross mice had been exposed to Sendai infection, but intercross mice were not. With backcross or intercross models, survival advantage favored heterozygous mice. We report herein the decline of T-cell responses and a higher incidence of lymphomas in *H-2^b* and *H-2^b/H-2^k* mice that explain the influence of the *H-2^b* haplotype to decrease life-span in *H-2* congenic mice.

MATERIALS AND METHODS

Animals. Female and male mice from parental strains B10 (*H-2^b*), B10.BR (*H-2^k*), and B.10.D2n (*H-2^d*) were obtained from The Jackson Laboratory. Mice were ear-tagged on arrival, and records of matings and offspring were kept. All F_1 hybrids obtained from parental strains were bred and housed in specific pathogen-free laboratories (Michael Redstone–Dana–Farber Cancer Institute). Mice were born between April 14 and July 28, 1987. The experiment lasted until February 2, 1991. Nine surveillance animals (DBA/2J females) were kept in each room at all times. Three or four of these were taken per month to test for infection by known pathogens. There was persistent evidence of *Pasteurella pneumotropica* (opportunistic infection), and, in four instances each, one or two surveillance animals were exposed to *Klebsiella* (opportunistic infection). The study animals, inspected weekly, showed no sign of infection, and no serological evidence of Sendai virus (parainfluenza virus 1) infection was observed. All mice, males and females, were housed separately in two rooms as described (5). The total number of mice analyzed was 1537. Mice were checked twice a day, 5 days a week, and once a day during weekends to ensure accurate determinations of dates of death and to facilitate necropsy analyses.

Cell Preparation. One hundred and twenty-five mice killed by cervical dislocation were used for immunological studies. Spleens were aseptically removed, and cells were suspended in chilled RPMI 1640 medium supplemented with 2 mM glutamine, 50 units of penicillin per ml, 50 μg of streptomycin per ml, and 10% (vol/vol) fetal calf serum. Single-cell suspensions were obtained by filtering spleen cells through nylon mesh. Erythrocytes were lysed by an osmotic method, and cells were washed twice with RPMI 1640 medium. Viability by trypan blue exclusion was consistently >95%.

Mitogenic Responses. Lymphocytes obtained from spleens were adjusted to 5×10^6 cells per ml, and 100 μl were plated in 96-well plates (U bottom) and incubated with 3 μg of phytohemagglutinin (PHA; Sigma) per ml, 2 μg of concanavalin A (Con A; Sigma) per ml, or 50 μg of lipopolysaccharide (LPS; Sigma) per ml. [^{3}H]Thymidine incorporation was measured after 48 hr for PHA and Con A responses and after 72 hr for LPS responses. All cultures were in triplicate, and proliferation was expressed as the arithmetic mean. Results are expressed as differences in cpm of stimulated and unstimulated cultures. In each experiment three (*H-2^b/H-2^k*)F_1 6-month-old mice were used as controls to normalize the data. The mean cpm of the three controls was used as 100%, to which responses were compared.

Statistical Methods. Survival curves were estimated by the method of Kaplan and Meier (6) with differences assessed by the log-rank test (7). Of the 1537 mice, 130 animals were censored in all survival analyses because of accidental drowning of 5 and sacrifice of 125 for immunologic studies. Also, an

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Abbreviations: PHA, phytohemagglutinin; LPS, lipopolysaccharide; Con A, concanavilin A.

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additional 20 mice were sacrificed because they had wounds or middle ear infections. They were not censored because they were not expected to live beyond 1 week. Associations between frequency of disease and sex were measured by using Fisher's exact tests. One-way analysis of variance was used to assess differences of immunological functions among the six strains. Pair-wise, multiple-comparison procedures were performed to detect differences between mean values of immunological parameters for all strains using Bonferroni *t* tests (8).

Classification trees were prepared by the method of LeBlanc and Crowley (9). Survival times are repeatedly divided into subgroups for each prognostic factor at levels that identify the biggest difference in survival. For each factor, two subgroups are created, and these are divided further until no longer worthwhile for prognostic intentions. We evaluated gender and *H-2* of six different mouse strains. The value associated with each group is the corresponding mean of the martingale residuals for that group; smaller values indicate better survival.

RESULTS

Gender and Life-Span. Males lived longer than females, with median lives of 29.5 and 28.1 months, respectively ($P < 0.001$). Analysis of gender differences within the different genotypes confirmed that males outlived females in both homozygous strains and heterozygous F_1 hybrids except for $H\text{-}2^d$ homozygous mice.

Effects of Reverse Breeding and *H-2* Genotype on Survival. Comparisons of reverse breeding of $H\text{-}2^k/H\text{-}2^b$ vs. $H\text{-}2^b/H\text{-}2^k$, $H\text{-}2^b/H\text{-}2^d$ vs. $H\text{-}2^d/H\text{-}2^b$, and $H\text{-}2^k/H\text{-}2^d$ vs. $H\text{-}2^d/H\text{-}2^k$ showed no differences in life-span attributable to such mating (data not shown). Therefore, analyses were performed after combining F_1 hybrids produced by breeding the different *H-2* congenic mice in both directions—i.e., $H\text{-}2^d/H\text{-}2^k$ was combined with $H\text{-}2^k/H\text{-}2^d$. Hybrids are named by *H-2* alleles—e.g., *b/k, b/d, d/k.*

***H-2* Haplotypes and Life-Span in Homozygous and Heterozygous Combinations.** Influence of *H-2* on survival is shown in Table 1. Among homozygous mice, the shortest life-span was in $H\text{-}2^b$ females (median, 26 months) and the longest survival was in $H\text{-}2^d$ males (median, 30.3 months). When one compares homozygotes and heterozygotes, the shortest lived mice were $H\text{-}2^b$ homozygotes and $H\text{-}2^b/H\text{-}2^k$ males or females, and the longest lived were $H\text{-}2^d/H\text{-}2^k$ males (median, 31.6 months). The $H\text{-}2^d$ and $H\text{-}2^k$ homozygous mice lived longer than $H\text{-}2^b$ homozygotes of both sexes. Combined life-span for the two groups with shorter life-spans, $H\text{-}2^b$ and $H\text{-}2^b/H\text{-}2^k$, was shorter than the combined life spans of the remaining four groups in Fig. 1, suggesting that the $H\text{-}2^d$ haplotype confers a survival advantage that is inherited as a dominant trait.

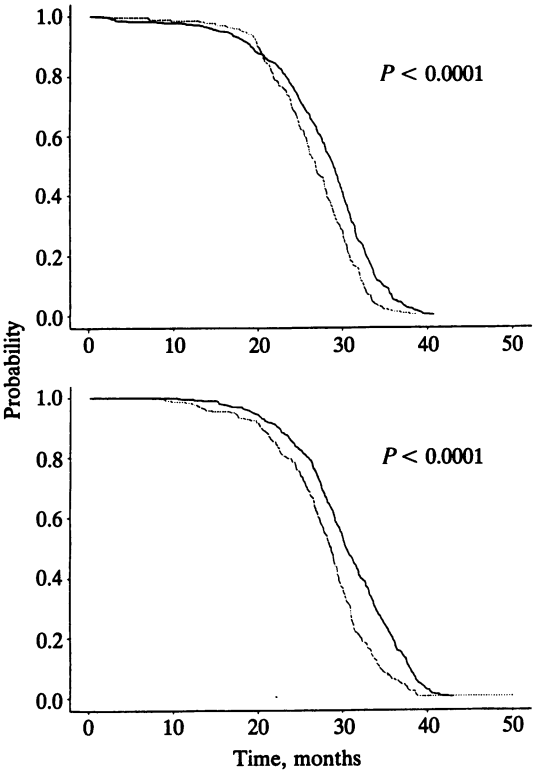

FIG. 1. Shorter-lived strains $H\text{-}2^b$ homozygotes + $H\text{-}2^b/H\text{-}2^k$ were combined and compared with the other four strains. (*Upper*) Curves for females of *H-2* haplotypes *b* + *bk* (252 mice) (broken line) and *d* + *k* + *bd* + *dk* (512 mice) (solid line). (*Lower*) Curves for males of *H-2* haplotypes *b* + *bk* (239 mice) (broken line) and *d* + *k* + *bd* + *dk* (404 mice) (solid line). The difference was significant for both sexes.

Tests of sex and strain showed significant differences ($P < 0.001$), and for each sex a difference within strains ($P < 0.001$ for both) was observed.

Heterozygosity Index. To associate heterozygosity with life-span, we calculated heterozygosity index as follows: one point for females and one for heterozygosity at *H-2*, with the maximum value = 2 and the minimum value = 0. Unexpectedly, the relationship between heterozygosity and longer life-span was inverse ($P < 0.001$): homozygous mice lived longer than heterozygous mice. The only heterozygotes for *H-2* that lived longer than homozygotes were $H\text{-}2^b/H\text{-}2^d$ compared with

Table 1. Group characteristics and life-span percentiles

H-2 genotype	Sex	Mice, no.	Life-span percentiles 10%	25%	50%	75%	90%	Ranks of median	95% CI		Log-rank *P* value
b	M	106	21.4	25.2	28.8	31.0	33.6	5	27.7	29.4	0.005
	F	104	19.9	23.2	26.0	30.0	32.4	6	25.4	26.8	
d	M	55	21.4	27.8	30.3	36.3	38.3	2	28.1	31.4	0.706
	F	45	16.8	23.5	29.5	35.4	38.3	1	27.6	29.8	
k	M	104	21.9	25.6	29.6	33.5	37.2	3	28.9	33.4	0.008
	F	123	21.2	24.4	29.1	31.6	33.5	2	27.0	31.5	
bk	M	162	19.9	24.4	28.1	31.5	34.4	6	27.0	29.3	0.011
	F	153	19.7	23.2	27.7	30.6	32.6	5	26.0	28.3	
bd	M	128	20.2	26.4	29.5	33.9	37.2	4	28.3	31.2	0.007
	F	123	19.4	24.5	29.1	31.5	34.5	2	27.9	29.9	
dk	M	193	23.7	27.4	31.6	35.6	38.4	1	30.2	32.7	<0.0001
	F	241	18.5	24.3	28.5	31.7	34.8	4	27.5	29.2	

Data show the number of months at which a certain percentage of the animals had died. The 50th percentile represents the median life-span in months, and the 90th percentile is the age in months at which 90% of the animals had died. The log-rank test compares the entire survival curve of males and females of each strain and not just the medians. CI, confidence interval.

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both male and female $H\text{-}2^b$ homozygotes (Table 1). The $H\text{-}2^d$ homozygotes lived longer than F_1 hybrids ($P < 0.01$), but differences between $H\text{-}2^k$ homozygotes and $H\text{-}2^d/H\text{-}2^k$ or $H\text{-}2^b/H\text{-}2^k$ were not observed.

Effects of Sex and *H-2* Genotype on Breeding Behavior. Mice were bred over 2–3 months beginning at 5–8 weeks. $H\text{-}2^d$ homozygotes were difficult to breed. Of these litters, 50% were lost before weaning, and mean litter size of conserved litters was only 2.9 ± 1.9. Of nine strains, the percentage of viable litters studied ranged from 98% of $H\text{-}2^d/H\text{-}2^k$ and $H\text{-}2^k$ homozygous strains to 51% for the $H\text{-}2^d$ homozygotes. No association between larger litter and survival was seen. Instead, $H\text{-}2^d$ homozygous mice that had a longer life-span showed the smallest litter size and the highest ratio of litters lost before weaning. These results differed from an earlier report (12).

Classification of Genotypes Based on Life-Span. Classification of gender and genotypes of censored and noncensored mice is illustrated by the use of CART (classification and regression trees) (Fig. 2), which provide a quantitative assessment of failure rate based on sex and strain. Gender represented the greatest difference among any two subgroups. As in Table 1 for both males and females, the first split separated the $H\text{-}2^b$ and $H\text{-}2^b/H\text{-}2^k$ strains from the remaining four.

Pathologic Findings. Of the total mice, 1028 mice (588 females and 440 males; 68% of the total) were necropsied. Remaining mice were too autolyzed to give useful information. Malignancies were more frequent (679, 66%) than other diseases. Lymphoma was a more common finding in females than males (46% vs. 36%) ($P < 0.001$). Other malignancies were more frequent in males: hemangiosarcoma (16% vs. 6%), hepatoma (8% vs. 3%), and lung adenocarcinoma (3% vs. 0.7%) ($P < 0.001$). Significant age differences were recorded for lymphoma vs. hepatoma, hemangiosarcoma, or lung adenocarcinoma. Median age for lymphomas was 26 months; hepatomas and hemangiosarcoma, 28 months; and lung adenocarcinoma, 32 months. Differences remained significant when analyzed by gender. Nonmalignant diseases, amyloidosis, glomerulonephritis, focal liver necrosis, vasculitis, pneumonia, colitis, pancreatitis, and pyelonephritis were <5% with no differences between sexes.

Table 2 illustrates relationships between strain, gender, and incidence of lymphomas. Strain differences in lymphoma incidence were present for both males and females. $H\text{-}2^d$ had a low incidence (24%), while $H\text{-}2^b$ had the highest incidence (54%). These findings were consistent with survival advantage of mice carrying the $H\text{-}2^d$ haplotype. Strain was not correlated with incidence of hepatomas or hemangiosarcomas.

Table 2. Incidence of lymphoma by *H-2* haplotype and sex

H-2 genotype	Female mice			Male mice			Total incidence	
	No.*	%	P†	No.*	%	P†	No.*	%
b	51/88	58		33/67	49		84/155	54
k	42/88	48		28/61	46		70/149	47
d	10/29	34		5/33	15		15/62	24
bk	66/123	54		43/111	39		109/234	47
bd	35/90	39		26/73	36		61/163	38
dk	67/170	39		22/95	23		89/265	34
Totals	271/588	46	0.0132	157/440	36	0.00075	428/1028	42

The percentage of animals with lymphoma is calculated over the number of animals in which postmortem examination was performed.
*Number of mice with lymphoma/total number of mice.
†The *P* values are a result of the test between the two different genders comparing the incidence of lymphoma among the six different strains.

Proliferative Response to PHA and Con A in Young and Old Animals. Responses to mitogens in old vs. young animals for each homozygous and heterozygous combination are recorded in Table 3, where significant decreases in T-cell proliferation in response to PHA were observed in old $H\text{-}2^b$, $H\text{-}2^k$, and $H\text{-}2^b/H\text{-}2^k$ animals as compared with young animals. $H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$, and $H\text{-}2^d/H\text{-}2^k$ mice each showed conserved vigorous proliferative responses at 27 months. Proliferative responses to Con A in $H\text{-}2^b$ and $H\text{-}2^k$ mice decreased with age as with PHA, but $H\text{-}2^b/H\text{-}2^k$, $H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$, and $H\text{-}2^d/H\text{-}2^k$ mice showed no decline of proliferative response with aging. Proliferative response differences were not observed among young animals of different strains or when the difference in cpm (Δcpm) was considered rather than normalized values (data not shown).

Proliferative Response Against LPS. LPS responses of B cells differed from PHA or Con A responses. No differences between responses of old and young animals in any strain or between responses of young and old mice between strains (Table 3) were seen.

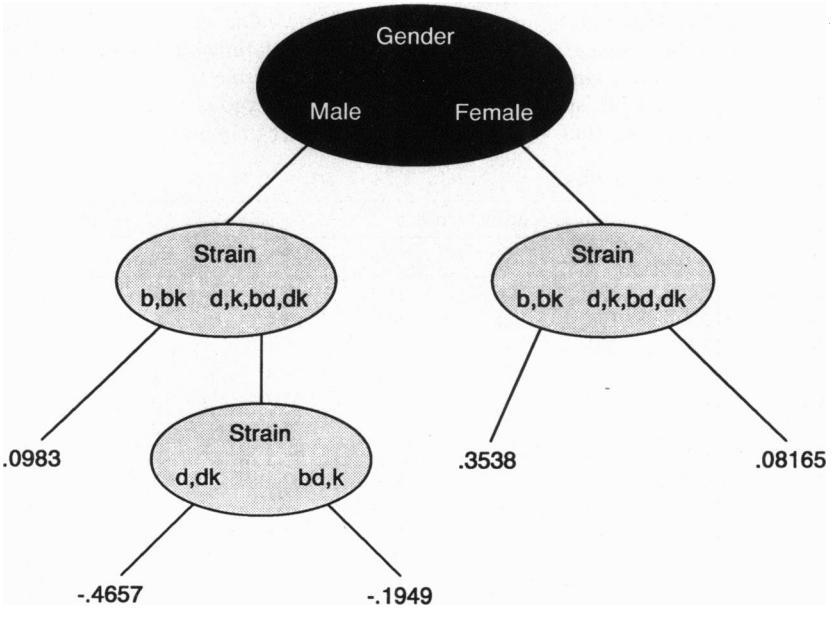

FIG. 2. Classification trees of six strains studied for males and females. The values associated with each group represent survival hazard; smaller numbers indicate better survival.

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Table 3. Proliferative responses of young and old animals

H-2 genotype	Young			Old			*P* value
	Mean cpm	SE	95% CI	Mean cpm	SE	95% CI	
			PHA				
b	39,095	6,476	55,744, 22,446	4,725	2,495	10,380, −1,380	0.0018
k	46,048	4,622	57,931, 34,166	12,863	7,025	30,054, −4,327	0.0183
d	41,983	6,547	58,005, 25,961	29,456	7,200	46,776, 11,536	0.2121
bk	46,220	6,177	65,881, 26,559	11,119	3,378	19,386, 2,851	0.039
bd	58,025	8,917	86,404, 29,646	36,025	9,657	59,665, 12,394	0.16
dk	53,609	7,964	76,501, 30,717	53,673	9,502	76,925, 30,420	0.37
			Con A				
b	42,158	6,815	58,834, 25,483	12,205	3,574	21,393, 3,016	0.0035
k	51,080	8,628	73,259, 28,901	23,418	5,641	37,921, 8,917	0.023
d	55,454	5,981	70,830, 40,078	42,645	9,395	65,635, 19,655	0.29
bk	45,403	4,205	58,784, 32,021	27,074	7,088	45,295, 8,853	0.088
bd	99,187	6,062	125,270, 73,103	67,672	11,263	96,914, 39,009	0.084
dk	118,974	12,501	172,761, 65,186	88,750	14,880	127,002, 50,498	0.16
			LPS				
b	38,107	5,326	55,058, 21,156	33,790	2,663	40,307, 20,359	0.43
k	39,706	5,843	58,303, 21,108	32,095	4,796	43,831, 20,359	0.35
d	38,215	1,929	44,354, 32,076	44,275	3,751	53,454, 35,095	0.28
bk	37,080	5,412	54,304, 19,855	34,639	4,879	46,578, 22,700	0.75
bd	62,913	2,899	72,140, 53,686	48,205	6,503	64,118, 32,292	0.14
dk	59,483	3,560	70,814, 48,153	54,072	3,798	63,365, 44,779	0.37

The mean and standard error from six experiments are shown. All of the strains were tested the same day. *P* value reflects the comparisons between young and old animals. CI, confidence interval.

Relationship Between *H-2*, Life-Span, Lymphoma Incidence, and T-Cell Responses. Comparisons of *H-2*, life-span, and T-cell responses are summarized in Table 4. With $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes in both homozygous and heterozygous combinations, the $H\text{-}2^d$-haplotype—homozygous $H\text{-}2^d/H\text{-}2^d$ or heterozygous $H\text{-}2^b/H\text{-}2^d$—mice yielded longer life-span, decreased lymphomas, and maintenance of vigorous T-cell responses with age.

$H\text{-}2^b$ and $H\text{-}2^k$ haplotype comparisons showed that, although the $H\text{-}2^k$ haplotype conferred survival advantage over the $H\text{-}2^b$ haplotype, this effect was observed only in homozygous $H\text{-}2^k/H\text{-}2^k$ mice. No differences in incidence of lymphomas or maintenance of T-cell responses between the $H\text{-}2^b$ and $H\text{-}2^k$ haplotypes were seen because both haplotypes were associated with frequent lymphomas and decreased immune function at 27 months.

Analysis of $H\text{-}2^d$ vs. $H\text{-}2^k$ haplotypes showed that the $H\text{-}2^d$ haplotype conferred survival advantage in females: $H\text{-}2^d$ homozygotes lived longer then $H\text{-}2^k$ or $H\text{-}2^d/H\text{-}2^k$ females. However, no difference in survival between $H\text{-}2^k$ and $H\text{-}2^d$ or between $H\text{-}2^k$ and $H\text{-}2^d/H\text{-}2^k$ males was evident. The incidence of lymphomas that correlated with the *H-2* haplotype was less in $H\text{-}2^d$ vs. $H\text{-}2^k$ animals: both females and males of the $H\text{-}2^d$ haplotype had fewer lymphomas than $H\text{-}2^k$ homozygotes.

DISCUSSION

Earlier studies revealed genetic interactions between genes on chromosomes 4, 19, and 17 and gender (5, 10). Interactions between gender and *H-2* haplotype varied from experiment to experiment, depending on exposure to Sendai virus infection.

Table 4. Summary of the role of *H-2* on life-span, incidence of lymphomas, and T-cell responses

$H\text{-}2_b$ vs. $H\text{-}2_d$		$H\text{-}2_b$ vs. $H\text{-}2_k$		$H\text{-}2_k$ vs. $H\text{-}2_d$	
Male	Female	Male	Female	Male	Female
		Survival			
$H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$ better than $H\text{-}2^b$ *d* vs. *b* $P = 0.001$ *b/d* vs. *b* $P = 0.002$	$H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$ better than $H\text{-}2^b$ *d* vs. *b* $P < 0.001$ *b/d* vs. *b* $P < 0.001$	$H\text{-}2^k$ better than $H\text{-}2^b$, $H\text{-}2^b/H\text{-}2^k$ *k* vs. *b* $P = 0.002$ *k* vs. *b/k* $P = 0.008$	$H\text{-}2^k$ better than $H\text{-}2^b$, $H\text{-}2^b/H\text{-}2^k$ *k* vs. *b* $P = 0.002$ *k* vs. *b/k* $P = 0.009$	$H\text{-}2^d/H\text{-}2^k$,* $H\text{-}2^d$,* $H\text{-}2^k$*	$H\text{-}2^d$ better than $H\text{-}2^k$, $H\text{-}2^d/H\text{-}2^k$ *d* vs. *k* $P = 0.02$ *d* vs. *d/k* $P = 0.007$
		Incidence of lymphomas			
$H\text{-}2^d$ lower than $H\text{-}2^b$, $H\text{-}2^b/H\text{-}2^d$ *d* vs. *b* $P = 0.001$ *d* vs. *b/d* $P = 0.042$	$H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$ lower than $H\text{-}2^b$ *d* vs. *b* $P = 0.06$ *b/d* vs. *b* $P = 0.009$	$H\text{-}2^b/H\text{-}2^k$,† $H\text{-}2^b$,† $H\text{-}2^k$†	$H\text{-}2^b/H\text{-}2^k$,† $H\text{-}2^b$,† $H\text{-}2^k$†	$H\text{-}2^d$, $H\text{-}2^d/H\text{-}2^k$ lower than $H\text{-}2^k$ *d* vs. *k* $P = 0.003$ *d/k* vs. *k* $P = 0.005$	$H\text{-}2^d$, $H\text{-}2^d/H\text{-}2^k$ lower than $H\text{-}2^k$ *d* vs. *k* $P = 0.4$ *dk* vs. *k* $P = 0.15$
		T-cell function			
$H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$ better than $H\text{-}2^b$ *d* vs. *b* $P = 0.024$§ *d* vs. *b* $P = 0.007$¶ *b/d* vs. *b* $P = 0.013$§ *b/d* vs. *b* $P = 0.002$§		$H\text{-}2^b$,‡ $H\text{-}2^k$,‡ $H\text{-}2^b/H\text{-}2^k$‡		$H\text{-}2^d/H\text{-}2^k$ better than $H\text{-}2^d$, $H\text{-}2^k$ *d/k* vs. *k* $P = 0.005$§ *d/k* vs. *k* $P = 0.003$¶ *d/k* vs. *d* $P = 0.0586$§ *d/k* vs. *d* $P = 0.06$§	

*All of them have long life-span.
†High incidence of lymphomas in all of them.
‡Low T-cell responses in all of them.
§Response to PHA.
¶Response to Con A.

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In two different experiments (5, 11) mice with the $H\text{-}2^b$ haplotype were longer lived than mice with the $H\text{-}2^d$ haplotype. However, exposure to Sendai virus was present in both experiments. F_2 mice typed as $H\text{-}2^d$ homozygotes or $H\text{-}2^d/H\text{-}2^b$ lived longer than the $H\text{-}2^b$ homozygous mice (10). Since the only difference between these and previously reported experiments was that mice in prior studies were exposed to and possibly infected by Sendai virus, we suggested that exposure to infection could change life-span. Susceptibility to this virus varies between strains (12), the most susceptible strain being $H\text{-}2^d$ (DBA/2). The $H\text{-}2^d$ haplotype confers susceptibility to the virus but, in the absence of infection, $H\text{-}2^d$ animals lived longer than $H\text{-}2^b$ animals. Herein we confirm that without Sendai virus infection, the $H\text{-}2^d$ haplotype confers longer survival than $H\text{-}2^b$. Further $H\text{-}2^k$ mice lived longer than $H\text{-}2^b$ mice. This finding differed from prior studies in which the $H\text{-}2^k$ haplotype ranked below $H\text{-}2^b$ for survival when mice were exposed to Sendai virus infection (13). Hybrid vigor was demonstrable only when comparing both males and females of $H\text{-}2^b/H\text{-}2^k$ vs. $H\text{-}2^b$ homozygotes.

Life-span in *H-2* divergent mice correlates with maintenance of immune vigor (3, 14). A slower rate of T-cell precursor decline has been correlated with long life (15). Herein immune function, *H-2* genotypes, life-span, and incidence of lymphomas were better correlated in males than in females. An exception, $H\text{-}2^k$ homozygotes exhibited a higher incidence of lymphomas than did $H\text{-}2^d$ homozygotes, and yet there were strains in which males had the longest life-spans. These findings are consistent with a prior analysis using leukemogenesis in *H-2* congenic mice due to BALB/Tennant-leukemia virus in which the most favorable allele was $H\text{-}2^d$ and the least was $H\text{-}2^b$ (16). $H\text{-}2^d$ conferred resistance in $H\text{-}2^d/H\text{-}2^k$ heterozygotes as compared with $H\text{-}2^k$ homozygotes.

Pathology at death and median life-span have been documented for some inbred strains (17). When *H-2* and tumor incidence in mice of C57BL/10 background was analyzed (18), lymphoma was the most frequent neoplasm in strains studied, and this tumor had a higher incidence in females. These findings differed from ours. We found $H\text{-}2^b$ mice to have a higher incidence of lymphomas than $H\text{-}2^k$ or $H\text{-}2^d$ mice. In several strains and F_1 hybrids, life-span and age-associated incidence of cancers were not significantly associated with genetic makeup. In F_1, F_2, and backcross mice of C57BL/6 and DBA/2 strains (5, 10), pathological findings could not be related to genotype, including *H-2*. A significant association between the incidence of lymphomas in both sexes of the $H\text{-}2^b$, $H\text{-}2^k$, and the $H\text{-}2^b/H\text{-}2^k$ mice was present when we autopsied >65% of mice that died. Further analyses will be needed to determine whether mice carrying the $H\text{-}2^b$ haplotype have premalignant clones of cells that become committed to produce neoplasia (19) and whether such cells may influence changes of T-cell functions later in life (1, 2, 15). Perhaps T-cell functions of aged mice relate to those of T cells in animals carrying tumors in which defective expression of $p56^{lck}$ and $p59^{fyn}$ and lack of expression of CD3γ are associated (20). Low T-cell responses with aging may reflect incipient malignancy or a tumor factor that alters function of T cells (21).

H-2 alleles influenced the vigor of immune responses in mice. Differences in proliferative responses to T-cell mitogens among young mice were not found. The mice homozygous for $H\text{-}2^b$ or $H\text{-}2^k$ exhibited a significant decrease with age in T-cell responses against PHA and Con A. However, $H\text{-}2^d$, $H\text{-}2^b/H\text{-}2^d$, and $H\text{-}2^d/H\text{-}2^k$ mice showed comparable proliferative responses between young and old animals, while old $H\text{-}2^b/H\text{-}2^k$ mice showed decreased proliferation vs. PHA responses, but Con A responses were comparable to those of young controls.

Does exposure of neonates to Sendai virus infection initiate different patterns of T-cell immune responses that lead to different responses of aged mice of different *H-2* genotypes? Endogenous retroviruses are known to be involved in immunoregulation and in development of some autoimmune diseases (22). Perhaps viral infections during life activate expression of retroviruses or expression of other molecules that directly or indirectly down-regulate immune functions associated with lymphomas later in life.

Life-span seems to be determined by interactions of environmental factors and several genes. Even when genetic differences exist, complex patterns of survival, immunological function, and pathology may be observed. This finding indicates that exposure to infection can change life-span—e.g., by alteration of T-cell functions. When mice were not exposed to Sendai virus, those of $H\text{-}2^d$ and $H\text{-}2^k$ haplotypes maintained T-cell function longer than mice of $H\text{-}2^b$ haplotype, and these parameters were correlated with survival, suggesting that the *H-2* haplotypes influence decline of important T-cell functions and thus of life-span.

Determination of life-span is complex, and one can anticipate that the influence of genes important to determination of life-span will require further analysis in the context of numerous genetic backgrounds and multiple environmental conditions. Even minimizing genetic and environmental variations, we can elucidate complex patterns of disease and survival and can demonstrate clearly the importance of certain major histocompatibility complex haplotypes in determining T-cell deficiency, incidence of tumors, and length of life.

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