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Genetics of life span in mice

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Abstract

Thymic involution that occurs earlier in some individuals than others may be the result of complex interactions between genetic factors and the environment. Such interactions may produce defects of thymus-dependent immune regulation associated with susceptibility to developing autoimmune diseases, malignancy, and an increased number of infections associated with aging.

The major histocompatibility complex may be important in determining profiles of cause of death and length of life in mice. Genetic influences on life span involve interactions between loci and allelic interactions during life which may change following viral infections or exposure to other environmental factors. We have used different experimental protocols to study the influence of H-2 on life span and found that interactions between genetic regions, are inconsistent, particularly when comparing mice infected or not infected with Sendai virus.

Genes important for life span need to be studied against many genetic backgrounds and under differing environmental conditions because of the complexity of the genetics of life span. Several genetic models were used to demonstrate that the MHC is a marker of life span in backcross and intercross male mice of the H-2^d and H-2^b genotypes in B10 congenic mice. Females lived longer than males in backcross and intercross mice, while males lived longer than females in B10 congenics. H-2^d was at a disadvantage for life span in backcross mice of the dilute brown and brown males exposed to Sendai infection, but intercross mice not exposed to Sendai virus of the same genotype were not at a disadvantage. H-2^d mice were not disadvantaged when compared to H-2^b in B10 congenics that had not been exposed to Sendai virus infection but the reverse was true when they were exposed. Overall, all our studies suggest that genetic influences in life span may involve interactions between loci and many allelic interactions in growing animals or humans. These genetic influences on life span may vary after they are exposed to infections or other environmental conditions. This paper emphasizes the need to use several genetic models, especially animals that have been monitored for infections, to study the genetics of life span.

Introduction

The potential life span of a species, defined as the duration of life of the longest survivors, is determined by genetic factors which control the rate of cellular and organ development and involution. It has been difficult to study the cellular and genetic factors influencing life span because the incidence of disease increases exponentially with age (Simms, 1946; Jucket & Rosenberg, 1988) and it is difficult to determine if the same mechanisms that mediate some diseases also control aging. Longevity of individuals within a species can also be determined by an absence of genetic susceptibility to diseases and the maintenance of the vigor required to live a normal life. Among the critical functions, a well-balanced immune system is necessary to cope with environmental stimuli and internal degenerative changes during life. The finding that life spans vary in different strains of mice suggested

that genetic markers for life span may be identified to explain such differences. Among the biomarkers of life span, the vigor of the immune system, which is under genetic control, has been thought to be important.

Theories concerning immunological dysfunction as a factor in aging have been stated by Walford (1969) and promulgated and developed by Burnet (1971). One theory holds that the immune system is essential for maintenance of health, and that its integrity determines survival advantage. Walford suggested that the breakdown in self recognition among cells by the immune system is secondary to genetic changes in somatic cells controlling immunity, resulting in the production of autoimmunity, malignancy, or increased susceptibility to developing infections.

The decline of vigorous immune function in aging humans or mice was found to be associated with the same diseases and immunological abnormalities found in individuals lacking T cells (Good & Yunis, 1974). Observations of this type led Burnet (1958) to suggest that clones of cells, ordinarily eliminated by the immune system, persist as immune functions become deficient with age. Immunodeficiencies, together with the persistence of such cells, may explain the autoimmunity which is frequently observed in both aging and immunodeficient individuals (Burnet, 1970).

Alternatively, others (Good & Yunis, 1974) interpreted these abnormalities in terms of a 'forbidden antigen' theory of autoimmunity, arguing that under immunodeficient circumstances, antigens otherwise excluded from the body or promptly eliminated are permitted to enter, to remain and to generate cross-reacting antibodies. They believe that a possible basis for an immunological theory of aging is based on thymic involution which can occur at varying ages (Yunis *et al.*, 1972). It was suggested that such a decline may be a genetically controlled 'clock' which operates at a rate consistent with the median life span of the species. Therefore, for the longest-lived member of a species, the 'clock' adheres to the limits imposed by the postulate of Hayflick (1965). It is unknown what mechanism controls the involution of the thymus. The genetic control of thymus involution is unknown but it may involve the CNS-endocrine system and not immune mechanisms *per se*. However, thymus involution could produce immune alterations and diseases that curtail life span, such as infections, neoplastic and autoimmune diseases, which could occur in immunodeficiency states as well as during aging (Roberts-Thomsen *et al.*, 1974; Hallgren *et al.*, 1978). Consistent with this explanation was the finding that some aged humans have an increased number of immunoglobulin-secreting cells and a deficiency of a regulatory T cell (Strelkauskas, Andrew & Yunis, 1981). This regulatory cell with the CD45 surface marker may be the cell necessary to provide the signal to increase the transduction defect of lymphocytes from aged mice and humans.

Table 1. Influence of age of donor of thymus and spleen and reconstitution of neonatally thymectomized mice.

Strain	Age of donor	8 month survivors	
		Thymus graft	Spleen cells
A	4 months	46% (28)	85% (34)
	12 months	52% (23)	87% (30)
	24 months	23% (13)	35% (26)
CBA	4 months	91% (11)	100% (15)
	12 months	92% (12)	91% (22)
	24 months	76% (17)	86% (21)

Total number of mice studies per group in parentheses.
Mice were treated intraperitoneally at 2 weeks of age with thymus grafts and spleen cells from mice obtained at different ages. From Yunis *et al.*, 1972.

Involution of the thymus and ensuing deficiency in cell mediated immune function occur earlier in certain individuals and inbred strains than others. For example, immunodeficiency observed in strain A mice during the second year of life is strikingly similar to that produced by neonatal thymectomy (Yunis, Fernandes & Stutman, 1971; Yunis *et al.*, 1972; Good & Yunis, 1974). Conversely, CBA mice, in which thymic involution is relatively late, tend to be long-lived and to maintain immunologic function longer than others. An experiment demonstrating that thymus involution occurs at earlier age in autoimmune susceptible strain A than a long lived strain (CBA) is shown in Table 1. Thymus grafts or spleen cells from CBA mice prevented death from wasting disease in neonatally thymectomized mice in a significant number of animals treated with thymus grafts from 4, 12 or 24 month old donors. The capacity of spleen cells obtained from A mice (known to be autoimmune susceptible and to have a short life span) prevented wasting

disease and death when the donors were 4 and 12 months of age but prevented wasting disease and death in only 35% when donors were 24 months of age. Thymus grafts from A mice prevented death from wasting less frequently, especially with donors at 24 months of age, than those grafted with thymus from CBA mice. These results showed that thymus involution and cells from the thymus-dependent system occur earlier in A stain than in CBA mice.

Neonatal thymectomy shortened the incubation period for development of antinuclear antibodies and immune complex glomerular disease in both the NZB and its (NZB × NZW)F1 hybrids. The spontaneous development of antinuclear antibodies in NZB and A mice (Teague *et al.*, 1970; Good & Yunis, 1974; Fernandes, Good & Yunis, 1977) was likewise facilitated by neonatal thymectomy. Such mice produced cell proliferation in the lymphoid tissues similar to that observed in aging mice of these autoimmune-susceptible strains. In contrast, CBA/H and C3H mice do not develop spontaneous autoimmunity during aging and showed decreased production of autoantibodies following neonatal thymectomy.

Neonatal thymectomy in autoimmune-susceptible mice produces several alterations: immunologic deficiencies, autoimmune hemolytic anemia, antinuclear and anti-DNA antibodies, and the hematologic, hepatic, splenic and renal lesions which appear early in life. These changes represent an acceleration of the processes associated with cellular immunodeficiency during aging in these strains (Yunis *et al.*, 1972). Table 2 summarizes one of these experiments. It shows that anti-DNA antibodies are found with higher frequency at 12 months of age in (NZB × NZW)F1 or NZB strains which are genetically susceptible to developing autoimmune diseases; the A, NZW and (A × NZW)F1 strains developed these antibodies later and with lower frequency. Further, C3H and CBA/H were strains resistant to developing autoimmunity and did not develop anti-DNA antibodies even at 18 months of age.

Attempts to correct immunodeficiency in thymectomized animals provided another line of evidence linking immunodeficiency to autoimmunity. In neonatally thymectomized mice both immunodeficiency and autoimmunity could be prevented by transplantation of thymus or spleen lymphocytes from syngeneic, semiallogeneic or allogeneic young donors (Good & Yunis, 1974; Fernandes, Good & Yunis, 1977). Even after wasting disease and autoimmune processes have appeared, effects in neonatally thymectomized mice were reversed by treatment with multiple thymus grafts or by injections of large number of thymocytes or peripheral lymphoid cells (Fernandes, Good & Yunis, 1977; Yunis *et al.*, 1972).

In summary, thymic involution is associated with the progressive decline of immune functions (Fernandes, Good & Yunis, 1977; Hirokawa, 1977). Although immune functions are important in the aging process, there are other functions that are equally important. Autoimmune diseases, infections and neoplastic diseases increase dramatically as the immune functions decline; such diseases are hallmarks of the aging process. As will be dis-

Table 2. DNA antibodies in different strains of mice.

Strain	6 months		12 months		18 months	
	%	N° mice	%	N° mice	%	N° mice
(NZB × NZW) F1	41%	17	59%	17	*	
NZB	37%	32	57%	23	*	
Af	5%	20	19%	21	21%	19
NZW	5%	22	28%	18	*	
(NZB × A) F1	7%	13	25%	15	*	
(NZB X C3H) F1	0%	23	4%	23	*	
C3H	0%	14	0%	14	0%	9
CBA/H	0%	24	0%	22	0%	15

* All animals died before 18 months of age; % indicates percentage of mice with DNA antibodies.
From Yunis *et al.*, 1972.

cussed in this chapter, other genetic systems may be important markers of life span. Environmental influences can also accelerate or delay this natural process. For example, immune abnormalities of aging can be restored by caloric restriction (Fernandes, Good & Yunis, 1977). Viral infections can also alter the genetic interactions which probably influence the immune changes that occur later in the life of some strains of mice.

Genes that affect life span in relation to the immune system

Variable life spans of pure inbred strains suggest that there are genetic factors that may be responsible for this variation. In regard to the role of genes in life span, many reports have shown that females live longer that males and that genetic defects that cause immunodeficiency shorten the life span of humans. Immune dysfunctions can be found in mice with mutations of genes in chromosomes X, Y,1, 2, 3, 4, 5, 6, 10, 11, 13, 14, 15, 16 and 19. These mice are commercially available but life span studies in them have not been performed (Shultz, 1993). Also, MHC alleles are important in immune responses and certain alleles may be involved in the control of autoimmunity and susceptibility for malignancies, which may indicate that certain MHC alleles may be associated with longer life span. We believe that certain MHC profiles are associated with shorter life span and others with longer life span, and that the genetic interactions involved differ when the animals are raised under different environmental conditions. The involvement of the MHC in such interactions which influence the development of malignancy and autoimmune disseases in aged individuals warrants further discussion.

MHC and malignancy

The first experimental evidence implicating the MHC in disease came from experimental work in mice which suggested that certain H-2 types mediated susceptibility to developing leukemia. In the case of Gross leukemia, H-2^b strains are resistant (Lilly, Boyse & Old, 1964). These results stimulated the studies of MHC associations with malignancy (Amiel, 1967). In other studies, increased tumor resistance of F1 hybrids to parental strain tumors was a manifestation of MHC linked genes, but such genes were not exclusively in the I region of the mouse H-2 complex and thus included genes that are distinct from the presently identified H-2 linked immune response genes. HLA associations to particular malignancies may represent survival differences rather than tumor incidence differences. This is a particular problem because of the necessary retrospective character of the HLA and malignancy studies. The most striking examples to be noted are an excess of HLA-A2 in long term survivors of acute lymphatic leukemia and the excess of HLA-A19 and HLA-B5 among short-term survivors of Hodgkin's disease (Simons & Amiel, 1977; Falk & Osoba, 1977).

Studies in mice suggested an influence of the MHC on natural killer cell activity as well as the possibility that natural killer cell activity influences the *in vivo* behavior of transplanted tumors (Williams & Yunis, 1978). Natural killer cell activity was elevated in F1 hybrids and controlled by MHC genes, the Hh genes (Petranyi *et al.*, 1976). HLA-B12 individuals had relatively high levels of natural killer cell activity (Simons & Amiel, 1977). Also, patients with acute myelogenous leukemia responded better to chemotherapy when they had the HLA-B12 phenotype (Parrish, Heise & Cooper, 1977; Pross & Baines, 1976). Thus, MHC genes as well as Hh genes may control the levels of NK killing and may explain HLA associations with survival of patients with AML.

The cell-mediated defect in mice or patients carrying tumors may not only involve NK cells but also T cells or soluble factors produced by them. Cancer patients and tumor-bearing mice demonstrated deficient immune functions manifested by decreased cell-mediated-immune assays and decreased lymphocyte cytotoxicity (Broder & Waldman, 1978; Hersch & Openheim, 1965; Takasugi, Ranaseyer & Takasugi, 1977; Young *et al.*, 1972). Furthermore, several mechanisms have been proposed to explain the immune impairment in cancer-carrying animals. These included production of suppressor cells, suppressor factors by tumor cells, deletion of tumor-specific clones and diminished production of cytokines (North & Bursuker, 1984; Webb, Morris & Sprent, 1990; Fearon *et al.*, 1990).

Association of MHC and autoimmune disease

HLA antigens have been found associated with

many diseases, a significant number of which are autoimmune in nature. In Caucasians a large number of diseases such as Insulin Dependent Diabetes, Rheumatoid Arthritis, several endocrinopathies, and Pemphigus Vulgaris (PV) were associated with class II MHC gene products carried by haplotypes marked by either HLA-DR3 or HLA-DR4. In autoimmune diseases characterized by autoantibodies, several genetic factors including the MHC, in addition to environmental factors, may be important in pathogenesis. An example of the role of the MHC and environmental factors in autoantibody production has been described in Pemphigus Vulgaris. This autoimmune disease caused by high concentrations of antibody to epidermal cadherin was associated with two kinds of HLA-DR4, DQ8 haplotypes dominantly inherited among Jewish patients, and these haplotypes plus DR6, DQ5 haplotypes in non-Jewish patients. Low levels of the PV antibody were found in asymptomatic family members. The inheritance of low levels of antibody in asymptomatic relatives has been linked to the MHC. Therefore, disease appears to occur in susceptible individuals with low levels of antibody when a second factor, either environmental or genetic, induces high levels of autoantibody sufficient to produce the disease (Ahmed *et al.*, 1993).

Association of MHC and life span

The MHC should influence life span, since it represents the main genetic factor regulating the immune system (Benacerraf, 1981). Evidence in support of the role played by MHC in life span comes from studies of congenic mice with three different strain backgrounds. Despite definite background-dependent differences in longevity when the H-2 allele was the same, distinct differences appeared among different H-2 congenics with the same background genome (Smith & Walford, 1977; 1978). The longest-lived strain with the C57BL/10 background, B10.R111, displayed the highest response to mitogens throughout most of life. The shortest-lived strain, B10.AKM, had the lowest proliferative response (Meredith & Walford, 1977). B10.F mice ($H\text{-}2^n$) demonstrated immunodeficiency and were shown to be short-lived (Popp, 1978).

Associations of immune functions with genotypes and life span in humans have not been studied systematically, but it has been shown that women older than 70 years of age had a lower frequency of HLA-B8 than younger women or men (the work was performed at a time when anti-DR reagents were not available). Women of this phenotype had lower T cell proliferative responses beyond 70 years of age than young controls or men carrying that phenotype (Greenberg & Yunis, 1978). Evidence presented at the Eighth International Histocompatibility Workshop Conference demonstrated association of HLA-B7 with Alzheimer's disease (Walford & Hodge, 1980) and association of HLA-DR1 with Xeroderma pigmentosum (Hodge, Degos & Walford, 1980).

MHC alleles are markers of long life span

Genes that may be markers for shortened life span have been identified. It is more difficult to identify genetic markers of long life. One example in mice was the association of longer life span in $H\text{-}2^b$ congenic strains when they were compared with congenic mice of other H-2 haplotypes. Within congenic mice, certain H-2 alleles significantly influenced life span but the same alleles may have comparatively different effects on life span on different backgrounds. On C57BL/10 and C3H backgrounds $H\text{-}2^b$ tended to mark long survival, on A strain to short survival. (Smith & Walford, 1978). Therefore, the use of congenic strains for genetics of life span may not be useful in understanding the genetic interactions that may be involved in the many aspects of life span in outbred mice. Analysis of HLA distribution in aged normal individuals showed an increased HLA-DR heterozygosity (Hodge & Walford, 1980). More importantly, subjects over 90 years of age had a low frequency of HLA-DR9 and increased frequency of HLA-DR1. The authors argued that a high frequency of HLA-DR9 and a low frequency of HLA-DR1 are associated with autoimmune or immunodeficiency diseases, which indicated that genetic protection against them may contribute to longevity (Takata *et al.*, 1987).

Experimental models to study genes of life span

The primary focus of this paper is the analysis of murine genetic research using four different genetic models to show possible gene variants influencing life span and the emphasis of the importance of the genetic interactions affecting life span in the exposure of mice to infections.

Table 3. Proportional hazards regression models and linear regression models of genetic markers.

Chromosome	Statistically indistinguishable Markers	Allele associated with longer survival	Significance level in final proportional hazards model	Significance level in final linear model
7	P450			
	Coh	B	0.0011	N/A
	Xmmv-35			
2	Ly. 24	B	<0.0001	0.0006
2	B2m			
	H-3	D	0.0006	0.0012
1	Lamb-2	D	<0.0001	0.0006
1	Ltw-4	B	<0.0001	<0.0001
12	Igh-Sa4			
	Igh-Sa2			
	Igh-Bg1			
	Igh-Nbp	D	0.0018	<0.0001
	Igh-Npa			
	Igh-Gte			
	Odc-8			
	Ox-1			
	Npid			
12	D12Nyu1	D	N/A	0.0011

Markers are indistinguishable if their genotypes were not known to differ in any of the twenty strains in the model.
From Gelman *et al.*, 1988.

Experiments using Recombinant Inbred strains (RI)
Recombinant Inbred strains have been shown to be useful in the analysis of segregation and linkage of multifactorial traits (Blizard, 1992).

Twenty strains of B × D RI mice (Taylor, 1989), composed of 395 females obtained from the Jackson Laboratory, were studied. No male mice were included in these studies (Gelman *et al.*, 1988). At the time of receipt at the Michael Redstone Animal Facility of the Dana-Farber Cancer Institute, the mice were between five and ten weeks old. They were representative of the 23 B × D RI strains available in 1982. Fifteen of the strains analyzed included 19 to 21 mice and five included nine or ten mice. Care was taken to minimize environmental influences such as temperature, noise, humidity, cage location, etc.

Published strain distribution patterns were available for up to 141 genes (actually, markers of an area of a chromosome) which were identified as being from the long-lived B or short-lived D parent. Most of these strains did not have data on several genes (For example: *Ly-22* on chromosome 4, *Saac* on Chromosome 9, *Igh-Bgl, Igh-Npa* on chromosome 12, and *Lyb-7*). Strains 2 and 14 were associated with significantly shorter life span, and strain 19 was significantly associated with longer life span. Four additional strains were associated with longer life span than the other 13 strains studied using a proportional hazards model of survival. The mean survival of the shortest lived strain was 479 days and the mean survival of the longest lived strain was almost double (904 days). Ranges of survival within strains were large (average of 642 days) and strains accounted for only 29% of the variation of survival; there were important environmental effects on life span, even in a colony housed in a single room. 101 markers of 15 chromosomes had distinguishable distributions on the 20 strains. The single region most significantly correlated with survival (marked by *Coh, Xmmv-35* on chromosome 7) divided the mice into two groups with survival medians which differed by 153 days (755 days for mice with the B genotype and 602 days for mice with the D genotype). It was expected that the genes of the B parent (using a proportional hazards or a linear regression model analysis) were better predictors of longer life span, but in general this was not corroborated.

Two different statistical models were used to

identify genetic regions of life span as shown in Table 3. The significance of the linkage between different genes in our studies was based on the use of proportional hazards and a linear model (Peto & Peto, 1972; Cox, 1972) using least squares and analyses of variance (Draper & Smith, 1966) and the R-squared and Cp with algorithm (Furnival & Wilson, 1974) with p values ranging between <0.001 and .001. Others consider that statistical evaluation of RI strains is based on Bonferroni's corrections for multiple testing (Neuman, 1992). It is quite possible that these genetic regions will not be as important predictors of life span as other genetic polymorphisms which were unknown at the time of the analysis (Gelman *et al.*, 1988). Future experiments using other RI mice are necessary to determine if these findings are generalizable. Also, male RI mice may show different genetic profiles for life span than the profile described for females.

Backcross study

Backross mice [(C57/6 × DBA/2)F1 × DBA/2] demonstrated that several chromosomal regions and the environment act together to influence life span in mice (Yunis *et al.*, 1984). The Cox model, used to test for interactions between the different variables, revealed two significant three-way interactions. ($H\text{-}2^b \times H\text{-}2^d$) F1 lived longer than $H\text{-}2^d$ homozygous in animals heterozygous for the *Brown* locus b (B/b) or homozygous (b/b). It was found that backcross mice produce different genetic life span profiles in relation to coat color markers and H-2 genotype. These backross mice showed primarily two genetic effects, the major histocompatibility complex in males and the coat color markers in females. Analyses of the *b* locus in relation to life span showed that the Bb mice lived longer than bb females, but the *dilute* locus (d) on chromosome 9 did not influence life span. The genetic interactions demonstrated that the *dilute* locus and the brown coat color have shorter life span in females. More importantly, there was a strong heterozygosity effect influencing life span. The longest-lived mice were females heterozygous at the *H-2* and *Brown* (b) loci. The shortest-lived mice were males homozygous at the *H-2* and *Brown* loci.

Intercross study

Analysis of genetic interactions in the F2 in an intercross of (C57BL/6 × DBA/2) F1 revealed influences of genetic factors on life span (Table 4). Females lived longer than males. *Dilute* brown females died sooner than females of other colors. $H\text{-}2^b/H\text{-}2^b$ males died sooner than $H\text{-}2^d/H\text{-}2^d$ or $H\text{-}2^b/H\text{-}2^d$ males, except that among *dilute* brown males those of type $H\text{-}2^b/H\text{-}2^d$ died sooner. Cluster analysis suggested that male and female genotypes each fall into two groups. Female *dilute* brown

Table 4. Group characteristics (life span in months).

Genotype		N° of mice		Ranks of medians		Median life span (95% confidence interval)		Log rank P-value
H-2	Coat color	F	M	F	M	Females	Males	
bb	*[B.][D.]*	152	157	6	9	27.3 (26.3, 27.6)	25.6 (24.8, 26.0)	0.003
bb	*[B.][dd*	46	58	8	11	26.5 (25.8, 28.0)	24.7 (24.2, 25.0)	0.157
bb	*bb[D.]*	65	36	7	10	26.7 (26.2, 27.2)	25.4 (23.9, 26.2)	0.0071
bb	*bbdd*	19	15	12	5	24.1 (23.9, 24.6)	26.8 (23.3, 28.5)	0.279
bd	*[B.][D.]*	301	294	2	4	28.4 (27.9, 28.7)	26.8 (26.4, 26.9)	0.0149
bd	*[B.][dd*	114	93	9	6	26.3 (25.7, 27.3)	26.4 (26.0, 27.3)	0.0923
bd	*bb[D.]*	90	114	3	2	28.1 (26.9, 28.5)	27.2 (25.8, 28.2)	0.0241
bd	*bbdd*	37	27	10	12	25.9 (25.3, 26.5)	24.6 (23.3, 26.2)	0.379
dd	*[B.][D.]*	158	162	4	3	27.9 (27.5, 28.4)	26.9 (26.6, 27.4)	0.113
dd	*[B.][dd*	50	53	1	7	29.3 (28.1, 30.2)	26.2 (25.3, 27.9)	0.021
dd	*bb[D.]*	47	43	5	8	27.8 (27.2, 28,3)	26.1 (24.6, 27.9)	0.0658
dd	*bbdd*	16	11	11	1	24.7 (23.2, 27.1)	27.6 (26.8, 28.9)	0.0318

Coat colors: [B.][D.] = black, [B.]dd = dilute black, bb[D.] = brown, bbdd = dilute brown. Brackets indicate phenotypes. Values are for comparisons of sex in each of the different genotypes. From Dear *et al.*, 1992.

mice have shorter lives than other females and male (H-2^b/H-2^b)F1 mice. *Dilute* brown females lived shorter life span than females of other genotypes regardless the H-2 genotype. *Dilute* brown (H-2^b/H-2^d) and H-2^d homozygotes lived longer than the H-2^b × H-2^d)F1. The remaining H-2^d homozygotes and (H-2^d × H-2^b)F1 males lived longer lives than H-2^b homozygous mice. The association of heterozygosity with life span was clearer in females than in males, yet the longest-lived female genotype was homozygous H-2^d/H-2^d of dominant black phenotype at the *Brown* locus of chromosome 4 and homozygous dd at the *Dilute* locus of chromosome 9. The shortest-lived females were *Dilute* brown H-2^b/H-2^b. The longest and shortest-lived male genotypes were *Dilute* brown H-2^d/H-2^d and *Dilute* brown H-2^b/H-2^d, respectively. (Dear *et al.*, 1992).

H-2 congenic study

Ten congenic strains of mice were studied for life span. It was possible to compare the K-end and D-end of *H-2* haplotypes. B10, B10.2R and B10.4R are H-2^b at the D-end; B10.A, B10.5R, B10.HTT, B10.T6R and B10.D2N are H-2^d at the D-end. B10, and B10.5R are H-2^b at the K-end; B10.D2^n H-2^d at the K end. H-2^k and H-2^s haplotypes were also examined on the B10 background. There was no influence of the K-end of H-2 in life span, but the differences at the E alpha in six of eight strains and at the D-end in eight of eight strains influenced life span. Another important finding was that the H-2^d haplotype was associated with shorter survival when compared to the other three H-2 haplotypes studied which included H-2^b (Gelman *et al.*, 1990).

New experiments supporting the association of MHC with immune responses, life span and development of malignancy

Analysis of *H-2* (H-2^b, H-2^k and H-2^d) haplotypes in congenic strains of mice and their hybrids revealed the unexpected finding that males lived longer than females in H-2^k and H-2^b homozygous and the three heterozygous combinations. H-2 interactions with gender demonstrated that the influence of H-2 in life span was found primarily in males of the H-2^d and H-2^k haplotypes. The association of heterozygosity with longer survival was evident only when comparing mice carrying the H-2^b haplotype with the (H-2^b × H-2^d)F1 hybrids. Males and female mice homozygous or heterozygous for H-2^d or H-2^k haplotypes lived longer than homozygous H-2^b or (H-2^k × H-2^b)F1 mice (Table 5, Salazar *et al.*, unpublished observations). H-2^d homozygotes and (H-2^b × H-2^d)F1 lived longer than H-2^b homozygotes (p = .001 in males or females). The incidence of lymphomas was lower in H-2^d homozygotes than H-2^b homozygotes (23% and 50% respectively, p = .001), or than (H-2^b × H-2^d)F1 (34%. p = .05). The incidence of lymphomas of H-2^b homozygotes was higher than that of the F1 hybrid (p = .009).

Role of viral infection in genetic interactions involved in life span

Mouse hepatitis virus infection is latent in many strains of mice. Neonatally thymectomized mice developed acute hepatitis, and this disease was

Table 5. H-2 congenics. Life span and group characteristics.

Genotype H-2	N° of mice		Ranks of median		Median life span 95% CI		Log rank P-value
	F	M	F	M	Females	Males	
b/b	104	102	6	5	26.0 (25.0, 26.8)	28.8 (27.6, 29.4)	0.0098
k/k	118	106	2	3	29.1 (27.3, 29.9)	30.0 (28.1, 31.6)	0.0049
d/d	45	57	1	2	29.5 (26.9, 31.5)	30.2 (28.5, 33.4)	0.84
bk	161	164	5	6	27.7 (26.0, 28.1)	28.1 (26.8, 29.1)	0.0078
bd	124	133	3	4	29.0 (27.6, 29.9)	29.5 (27.8, 31.2)	0.0044
dk	241	197	4	1	28.3 (27.5, 29.4)	31.7 (30.2, 32.7)	< 0.001

The median life spans are ranked separately for each sex. Confidence intervals are wider for smaller groups of mice. The log rank tests compare entire survival curves, not just the medians. P-values are for comparisons between males and females.

Table 6. Comparison between backcross, intercross and congenic life span studies.

Parameter	Backcross (C57BL/6 × DBA/2) F1 × DBA/2	Intercross (C57BL/6 × DBA/2) F1 × F1	B10 Congenic Exp 1	B10 congenic Exp 2
H-2 locus influence	Males	Males	Males and females	Males and females
H-2d short life in males	Yes	No	Yes	No
Sendai infection	Yes	No	Yes	No
b locus on chromosome 4: Bb better than bb in females	Yes	Not studied	N/A	N/A
d locus on chromosome 9: Dd not better than dd	Yes	Not studied	N/A	N/A
Longer life span	Females	Females	Males	Males
Genetic interactions	Dilute brown/brown have shorter life span in females	Dilute brown shorter life span in females	N/A	N/A
Heterozygosity effect	Strong	Relative	Relative	Relative
H-2d/H-2d	Disadvantaged in dilute brown males and females	Not disadvantaged	Disadvantaged	Not disadvantaged

primarily found in animals developing wasting disease (Stutman, Yunis & Good, 1972). Such studies suggested that T cells are required to prevent activation of latent viral infections.

In the case of Sendai virus infection, T cells and the major histocompatibility complex were involved in susceptibility to acute infection and early death (Stewart *et al.*, 1978), with highest incidence in H-2^d/H-2^d mice (DBA/2) (Parker, Whiteman & Richter, 1978). However, we believe that mice that were exposed to Sendai virus and survived the exposure to infection developed T cell defects detectable later in life. In the absence of virus infection or exposure to the virus, the H-2^d conferred longer life span than mice of H-2^k or H-2^d genotyopes.

In several studies summarized in Table 6, using different experimental models, significant genetic interactions between genes on chromosomes 4, 9, 17 and gender were demonstrated (Yunis *et al.*, 1984; Gelman *et al.*, 1988; Gelman *et al.*, 1990; Dear *et al.*, 1992). In experiments where the mice had been exposed to Sendai virus infection it was found that the H-2^b phenotype was associated with longer life span than was H-2^d or H-2^k (Smith & Walford, 1977; Gelman *et al.*, 1990). However, in other experiments using a different experimental model, it was demonstrated that mice H-2^d homozygous or (H-2^d × H-2^b)F1 lived longer than the H-2^b mice when they were not exposed to the same infection. The only important difference between the studies reported was that the mice had been exposed to and possibly infected with Sendai virus. These experiments suggested that exposure to infection can change the profile of life span (Dear *et al.*, 1992). Earlier findings that H-2 significantly influences life-span primarily in males (Yunis *et al.*, 1984) were corroborated. While the same loci remain important, it appears that environmental changes such as exposure to Sendai virus and the segregation of other genetic systems in different experimental models affected the details of genetic interactions. In the backcross experiments H-2^b/H-2^b animals lived longer than the H-2^d/H-2^d animals, suggesting an influence of heterozygosity on life span, but in the F2 experimental model the H-2^d haplotype conferred long life span. Furthermore, the heterozygosity index was associated significantly with life span in animals that had been exposed to Sendai. In the F2 experimental model heterozygosity *per se* did not

increase life span in mice. Although the association of life span with heterozygosity was significant in females, the longest-lived mice were females predominantly heterozygous for the *Brown* locus of chromosome 4, but homozygous for H-2^d/H-2^d and for the *Dilute* locus of chromosome 9.

It is noteworthy that Sendai infected congenic mice, which differed only at H-2, demonstrated that H-2^b mice lived longer than H-2^d mice. This finding was confirmed in backcross mice. In contrast, in the absence of Sendai infection H-2d was a better predictor of long life span than H-2b as demonstrated in intercross mice (Dear *et al.*, 1992). Unpublished experiments using congenic mice confirmed that in the absence of Sendai virus infection, H-2d mice live longer than H-2^b mice (Salazar *et al.*, unpublished). The studies also demonstrated that T cell functions were diminished in H-2^b homozygotes as compared to H-2^d homozygous or (H-2^b $\times$ H-2^d)F1 in males and females. It is not unusual that proliferative responses to lectins such as PHA are decreased in aged mice. What is different is that the defect was found more significantly in mice of H-2^b genotype.

In previous studies, histopathological findings failed to demonstrate a relationship between cause of death, genotype and life span (Salazar *et al.*, unpublished). This may be due to the fact that previous studies included autopsies at death in less than 25% of the animals as compared to more than 75% autopsies performed in our unpublished experiments using H-2 congenic mice and their hybrids. A higher incidence of lymphomas was correlated to H-2^b homozygous and (H-2^b $\times$ H-2^k)F1 when compared to a lower incidence in H-2^d homozygotes and (H-2^d $\times$ H-2^k)F1 mice.

Discussion

It has not been possible to determine a single gene that can predict long life in outbred populations of mammals. Different life spans in various inbred strains of mice suggested genetic influences which have been associated with specific diseases. In many experiments females lived longer than males and F1 hybrids lived longer than parental strains (Russell, 1975; Yunis *et al.*, 1984). We have summarized evidence in favor of genetic factors that influence immune responses during life span. It is remarkable that MHC influenced life span in several experimental models in the absence or presence of multiple genetic interactions. Nevertheless, studies also suggested that life span results from environmental factors interacting with several genes, which include the genes of the major histocompatibility complex.

With regard to the influence of H-2 alleles in the frequency of lymphomas in H-2 congenic mice, H-2^b was more susceptible to lymphomas in older animals than H-2^d mice of the same age. However, other studies had shown that H-2 alleles alone could not alter susceptibility to the genesis of lymphomas. Our studies demonstrated that H-2^d was associated with longer life span, maintained T cell immune vigor in old age, and had lower incidence of lymphomas. Current models for lectin-induced T cell proliferation suggest that activation of protein kinase C (PK-C) and elevation of cytoplasmic CA^+ may both play important roles in the earliest phases of signal transduction. T cells from young mice responded as well to optimal combinations of these agents as they did to the strong polyclonal activator Con A, but T cells from old mice responded better to optimal combinations of PMA (phorbol myristate acetate, a PK-C activator) plus calcium ionophore ionomycin than they did to Con A. This suggests that an inability to transduce the signal supplied by extracellular ligands into the intracellular signals represented by PK-C and CA^+ activators could be the underlying mechanism for age-associated loss of T cell reactivity. Furthermore, T cells from old mice required higher levels of ionomycin for maximal proliferation than cells from young animals (Miller, 1986).

Recently it has been reported that animals bearing a tumor longer than 26 days develop $CD8^+$ T cells with impaired cytotoxic function, decreased expression of the tumor necrosis factor-α and ganzyme β genes, and decreased ability to mediate an antitumor response *in vivo*. The structure of the TCR was evaluated by surface iodination of the purified T cells from normal and tumor-bearing mice. T lymphocytes from tumor-bearing mice expressed T cell antigen receptors that contained low amounts of CD3γ and completely lacked CD3ζ which was replaced by the Fcϵ γ-chain. Expression of the tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ was also reduced. These changes were interpreted to explain the basis of the immune defects in tumor-bearing

animals. These authors also reported that the same findings occur in human cancer patients whose peripheral blood T cells lacked expression of lck and Fyn protein. Thus, structural and functional signal transduction molecules from tumor-bearing mice and humans may explain T cell abnormalities in cancer bearing animals and humans (Mizoguchi *et al.*, 1992). They demonstrated that these clones are manifested clinically only in old age. Other authors showed that the immune defects found in murine hosts bearing tumors have a decrease in cytotoxic function of CD8-cells, suggesting one mechanism responsible for immunologic defects of CD8 effector T cells in patients with tumors (Loeffler *et al.*, 1992; Mizoguchi *et al.*, 1992). It seems that tumors can produce a factor that changes the functions of T cells. It is unknown if premalignant clones (of spontaneous tumors) *per se* can produce some of these alterations, or if other factors (environmental, such as infections) can produce similar changes, which would predispose animals or humans to develop tumors during aging (Newell, Spirtz & Sider, 1989). It is not known if the defect of T cell function found in aged mice and humans is identical to that found in T cells of animals carrying tumors, defective expression of $p56^{lck}$ and $p59^{fyn}$ and lack of expression of CD3γ in association with TCR. For example, changes in T cells of tumor-bearing mice may be found in aging mice, which might explain the immune defects associated with aging. Low T cell responses during aging may represent evidence that they have incipient malignancy or predisposition for malignant disease. It is possible that lymphomas seen late in life arise from premalignant clones of cells that have become committed to neoplasia in young animals (Van Houten *et al.*, 1989). The progressive growth of a subcutaneous implant of a tumor in mice resulted in decreased lytic function of the $CD8^+$T lymphocytes that was associated with decreased expression of mRNA for tumor necrosis factor-α and granzyme β, and the complete loss of the ability of adoptively transferred cells to mediate an antitumor effect *in vivo* (Loeffler *et al.*, 1992). In these experiments there was no detection of suppressor function. These results contrasted with early studies in the development of spontaneous tumors due to mammary tumor virus, where there was a proven role of suppressor cells in the tumor development (Fernandes, Yunis & Good, 1976).

The genetics of life span are complex and we anticipate that genes important in life span will need to be studied in many genetic backgrounds and environmental conditions. It is remarkable that even after minimizing the genetic and environmental variations we have found complex patterns of disease and life span, which raises doubts as to the feasibility of studying genetic effects of life span in outbred populations. At the very least, it would be necessary to identify genes that under all environmental conditions influence life span, a task difficult to undertake in the near future. Since the National Institute of Aging has recently undertaken an initiative to identify genes of life span in outbred populations of mammals, careful attention must be paid to the problem of identifying these genes for this initiative to be productive. We emphasized the importance of the thymus and T cells during the life of an animal and that thymus-dependent immunity prevents development of diseases common in aged individuals. MHC alleles may also determine susceptibility to disease but it is not known if its genetic influence can direct the involution of the thymus or the thymus-dependent immune functions. New research is needed to invesitage the role of environmental factors at different times in the life of an animal and the possible production of latency to developing diseases later in life. Areas of research that need intensive investigation include at least two:

a) Identification of premalignant clones of cells latent in animals susceptible to spontaneous malignancies.
b) Investigation of mechanisms of immune regulation that may occur following infections during the neonatal period, for example the role of endogenous retroviruses following exogenous viral infections or other infections (Talal, Flesher & Dang, 1992). These environmental factors may activate retroviruses or other molecules which could be involved in the development of immune dysfunctions and lymphomas, or other malignancies that develop later in life.

In other words, experiments are required to prove that the environment may be playing a more significant role in the immune or other functions of the growing animal than had been previously thought. In that case, genetic influences on life span would involve interactions between loci and many allelic interactions in the growing individual which may

vary after the individual is exposed to different infections or other environmental factors.

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