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INHERITANCE OF IMMUNE RESPONSIVENESS, LIFE SPAN, AND DISEASE INCIDENCE IN INTERLINE CROSSES OF MICE SELECTED FOR HIGH OR LOW MULTISPECIFIC ANTIBODY PRODUCTION

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High (H) and low (L) antibody responder lines of mice separated by selective breeding present a maximal interline difference in antibody (Ab) response to Ag of different specificities (general genetic regulation).

The analysis of SRBC agglutinin response in H line, L line, F_1 hybrids, F_2, and backcross segregants demonstrates that Ab responsiveness is a polygenic trait regulated by the additive interaction of 5 to 7 independent loci, with an incomplete dominance (44% ± 7%) of the high response character, and a 30% ± 10% impact of the environmental factors.

The life span of H, L, F_1, F_2, and backcross populations is correlated positively with 2-ME-resistant agglutinin response ($r = 0.97$, $p < 0.001$) and negatively with 2-ME-sensitive agglutinin response ($r = 0.95$, $p = 0.01$) (interpopulation correlation). Similar correlations are also observed in individuals of the various populations, especially in $F_1 \times L$ backcross, in which the largest phenotypic variance is found. The positive correlation between Ab responsiveness and life span was confirmed by ELISA titration for distinct IgG isotypes (intrapopulation correlation).

Malignant lymphomas and chronic nephritis were the two most common diseases observed. The age-adjusted incidence of such diseases, which is largely affected by environmental factors, accounts for the longer life span of H, as compared with L, mouse populations. The longevity of the 30% or less survivors, chiefly determined by the rate of physiologic aging, is a polygenic character regulated by the cumulative interaction of 3 to 7 independent loci, with a complete dominance of the long life trait and an impact of the environmental factors of about 60%.

Thus we have grounds for regarding general Ab responsiveness and life span as polygenic traits regulated by a small number of identical or closely linked gene loci, and immune responsiveness as a defense mechanism against neoplastic and inflammatory diseases.

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The major teleonomic function of the immune system is to confer on natural animal populations the optimum protection against all types of endemic and epidemic infections (1–3). Much less obvious, though, is the part played by immune reactions in antitumor resistance, although a great many experimental and clinical observations uphold the concept of an immune surveillance on initial proliferation of malignant cells (4, 5). These protective functions of the immune system are bound to have a significant impact on a population life span. Early studies in inbred mice demonstrate that life span and disease are controlled by the interaction of genetic and environmental factors (6–14). Life span is a polygenic character, and the number of loci involved does not seem large enough to preclude a genetic analysis (15–17). The incidence of changes in the immune functions of aged individuals led to the formulation of an immunologic theory of aging (18–20). This theory was strengthened up by the finding that the MHC containing the specific Ir genes (21) was also linked to genes affecting the incidence of various pathologic phenomena and life span (22–27).

Selective breedings of H[2] and L Ab responder lines of mice clearly demonstrated that immune responsiveness to natural polydeterminant immunogens, such as bacteria or heterologous SRBC and proteins, is a polygenically controlled character (general review in Ref. 1) in which MHC-linked genes play but a partial and irregular role (28).

Several selective breeding experiments were carried out from distinct F_0 of outbred mice, by using different immunogens and various immunization procedures. In all selections the effect was multispecific, the difference in Ab responsiveness between H and L lines being not restricted to the selection Ag, but common to several Ag of distinct specificities (1).

In both selections I and II, carried out from distinct F_0 populations for primary Ab response to heterologous E, H lines presented a markedly longer life span and a lower incidence of tumors than L lines (29, 30).

In order to produce homozygous lines at the levels of all the independent loci regulating Ab responsiveness, the selective breeding procedure was pursued over many consecutive generations, until the maximal interline separation was attained (selection limit). At that time each

[2] Abbreviations used in this paper: H, high responder line; h_2, realized heritability; L, low responder line; Ab, antibody; F_0, foundation population; 2-ME-R, 2-ME resistant; 2-ME-S, 2-ME sensitive; *CV*, coefficient of variation; *r*, correlation coefficient; $\bar{X}$, mean; *V*, variance; Bc, backcross.

line constituted therefore a closed colony presenting an inescapable increase in genetic homogeneity due to random drift (31). It was not clear whether the effect on life span and tumor incidence observed in selections I and II was (1) due to the genes involved in immunoregulation, or (2) to any fortuitous concomitance of genetic modifications elsewhere. But if ever a common inheritance pattern of these traits were found in segregant hybrids from H and L line crosses, the first (1) eventuality would then be confirmed.

In the present study we investigate the Ab response, life span, incidence of tumors and other diseases in H and L lines of selection II, in their interline F_1 hybrids, in the F_2 segregants and in the progeny of F_1 backcrossed with H or L lines.

MATERIALS AND METHODS

Mice. Selection II was carried out at the Institut Curie, Paris, France, for maximum (H line) or minimum (L line) serum agglutinin response to SRBC (32). The maximum interline separation (selection limit) was reached after 15 succedent generations (F_{15}) of selective breeding. The assortative mating was then pursued in the subsequent generations (F_{15} to F_{48}). H and L lines at selection limit may be considered as homozygous at the level of all loci participating in the regulation of Ab responsiveness.

Starting from parents of the F_{39} generation, colonies of H and L responder mice were established in the facilities of the Laboratory of Pathology at ENEA, Casaccia, Rome (Italy). The results presented in this article, apart from those in Table I, were obtained in mice produced at ENEA. The mice were housed at random, three to a cage, and maintained on standard pellet food plus water ad libitum. The animals quarters were kept at 20°C and a 60% relative humidity, on a 12 h light and 12 h dark cycle.

Antibody response. Male and female mice were immunized i.v. at 60 days old with the optimal dose of 5×10^8 SRBC. Blood was withdrawn from the retro-orbital sinus 7 and 14 days post immunization, and individual sera were titrated for total agglutinins and also, after 2-ME treatment (33), for 2-ME-R agglutinins. The 2-ME-S agglutinins were calculated by substracting the 2-ME-R titer from the total agglutinin titer. The agglutinin titer was expressed as the highest doubling ($\log_2$) serum dilution producing a positive SRBC agglutination. Isotype distribution of SRBC antibodies was evaluated on pooled sera titrated by the ELISA technique. Plates were coated with 4×10^6 SRBC/well, and fixed with 0.25% glutaraldehyde as described by Heyman et al. (34). Rabbit anti-mouse isotype sera, conjugated to β-galactosidase (IgG2a, IgG2b) or to alkaline phosphatase (IgG, IgG1, IgG3), were obtained from ZYMED (San Francisco, CA). Results are given as $\log_2$ titers for OD values equal to 30% of the optimal plateau level (H line value).

Life span and pathology. Mortality was scored daily (six checks a week) for the entire experiment. In a few cases, moribund mice were sacrificed. Soon after spontaneous death, complete autopsy was performed on 870 out of 878 mice (99%) under investigation. The necropsy included complete external and internal gross examination. Tissue masses as well as sections of the major organs were taken and processed for histologic analysis. Tissues were fixed in Bouin's fluid and processed for paraffin embedding and sectioning. Sections were stained with hematoxilin and eosin routinely; some sections were also processed for Congo red stain according to Bennhold's method, to ascertain the presence of amyloid substance.

Histologic diagnoses were coded and entered in a computer for statistical analysis.

Life span was measured in each population as the mean ($\bar{X}$) of male and female ± SD or SEM, median life span, mean age to death ± SD% of the 10% shortest lived mice (first percentage), of the 30%, 20%, and 5% longest lived ones (last percentages), and of the last two (one male, one female) survivors. The Weibull method (35) was used to model mortality distribution with time for all causes in the different populations. This model is a generalization of the exponential distribution, as it does not assume a constant hazard rate and therefore has a broad application. The cumulative mortality $M(t)$ as a function of time (t) is expressed as $M(t) = 1 - [\exp - (\lambda t)^{\gamma}]$ where γ and λ are the shape and scale parameters, respectively. The maximum likelihood estimates of γ and λ were obtained for each population by means of an iterative procedure. A two-sample test proposed by Thoman and Bain (in 35) for samples without censoring was used to compare mortality distributions. Disease occurrence was evaluated in terms of percentage of lesion-bearing animals (hereafter referred to as incidence), without or with age-adjustment for the differences in the mortality rate of the different populations, in accordance with the method described in detail by Ulrich et al. (36), modified to account for accidental losses during the experiment (37). Furthermore, age-related death rates for lesions diagnosed at death were computed and plotted as cumulative probabilities in function of time, according to the model of Rosenblatt et al. (38). This methodology analyzes both the frequency of a disease at death and the time of its occurrence by means of a single set of statistics, taking competing risks and losses into account; it is therefore useful for the comparison of the different experimental populations investigated.

Genetic analysis. The two polygenic characters "Ab response" and "life span" were analyzed according to the same models used in the previous studies on immune responsiveness (1), as described in detail by Falconer (39), and by Cavalli-Sforza and Bodmer (40).

The mean ($\bar{X}$) and variance (V) values of longevity and Ab response in each population were established from the individual life span in days, and from individual $\log_2$ agglutinin titers respectively. The $\bar{X}$ and V of life span for the first dead and last survivors were calculated from normalized distributions by including symmetrical data.

The following expressions were used. Additive effect: $a = (\bar{X}H - \bar{X}L)/2$. Dominance deviation: $d = \bar{X}F_1 - (\bar{X}H + \bar{X}L)/2$; dominance effect: d/a; phenotypic variance of F_2 segregant populations $VP\ F_2 = VA + VD + VE$; phenotypic variance of the two backcrosses (Bc): $BcH + BcL = VA + 2VD + 2VE$, where VA is the additive variance, VD the dominance variance, and VE the environmental variance.

VE was calculated as the mean of the phenotypic variance of the three genetically homogeneous populations: H, L, and F_1.

The percentage of the phenotypic variance (VP) determined by genetic factors (VG) was calculated in the interline segregant F_2 and Bc as: $VG = 1 - (VE/VP) \times 100$.

VA and VD values were calculated from the equation $VD/VA = 1/2(d/a)^2$.

Heritability of the character investigated (h_2) was estimated in F_2 segregants: $h_2 = VA/VP\ F_2$.

The number of gene loci (n) participating, by additive effect, in the quantitative regulation of the character investigated may be evaluated as: $n = a^2/2VA$. The n value should be considered as a segregation index estimating the number of chromosome segments containing the relevant genes which, being located far apart, segregate independently.

RESULTS

Ab responsiveness. As demonstrated in our previous studies (1), the individual $\log_2$ agglutinin titers have a normal frequency distribution in H and L lines and in their various interline crosses, which is verified in the present study. The mean and variance of Ab responses are thus correctly measured. The principal immunogenetic parameters established in homozygous H and L lines after selection limit ($F_{15} - F_{48}$) and in the progeny of their interline crosses produced at the Institut Curie are shown in Table I.

The results in Table I demonstrate the extremely large modification in Ab responsiveness produced by the selective breeding. The interline difference in the 14-day agglutinin response ($2a = 9.8$) corresponds to a 900-fold difference in terms of serum agglutinin titers (H = 1/7600, L = 1/8.5).

The character "high response" presents an incomplete dominance effect of 49% ($d/a = 0.49$).

Considering that the phenotypic variance of the genetically homogeneous H, L, lines and F_1 hybrid populations is produced by environmental effects, it may be calculated that approximately 55% of the phenotypic variance in segregant F_2 and Bc populations is due to the interaction of 5 to 10 independently segregating loci. The variance produced by the dominance effect (VD) accounts for approximately 10% of the genetic variance in the segregant populations.

The 14-day agglutinin responses of H and L mice orig-

TABLE I

Fourteen-day total SRBC agglutinin response in H and L responder lines and in the progeny of interline crosses bred at the Institut Curie, Paris, France[a]

Mice	No. of Mice	Total Agglutinin Titer	
		$\bar{X} \pm SD$	Variance (V)
H	950	12.9 ± 1.1	1.2
L	926	3.1 ± 1.3	1.7
F_1	241	10.4 ± 1.0	1.0
F_2	363	9.8 ± 1.7	2.9
BcH	262	10.5 ± 1.0	1.0
BcL	248	7.7 ± 2.1	4.4
$a = 4.9$	$d = 2.4$	$d/a = 0.49$	$VE = 1.3 \pm 0.14$
$VG\ F_2 = 55\%$		VG Bc = 52%	
$VA\ F_2 = 1.43 \pm 0.59$		VA Bc = 2.26 ± 0.59	
$VD\ F_2 = 0.17$		VD Bc = 0.27	
n Range = 6.9 – 10.6		n Range = 4.7 – 6.2	
$\bar{X}n = 6.9 \pm 2.2$			

[a] V BcH + V BcL = V Bc. For VE, VA, and n values: ± = SE. See text for other symbols.

inating from the F_{39} generation at the Institut Curie, and bred at ENEA, were: 13.2 ± 1.0 and 3.8 ± 1.1, respectively ($2a = 9.4$). These results, compared with those in Table 1, demonstrate that these two distinct environments did not significantly modify the expression of the genes involved in the regulation of Ab responsiveness.

A large part of the results in this article concerns the early (7 day) responses when 2-ME-S agglutinins can still be evaluated. The complete immunogenetic analysis of the 7 day responses in the mice produced at ENEA are presented in Table II. Table II shows the total and 2-ME-R agglutinin responses in H and L lines and in the progeny of their interline crosses. The main immunogenetic parameters have also been entered.

The total agglutinin level in H line increased from 11.4 to 13.2 during the 7 to 14 day post-immunization period, whereas it decreases from 5.2 to 3.8 in L line. As a result, the interline difference in the total agglutinin response is smaller on day 7 ($2a = 6.2$) than on day 14 ($2a = 9.4$).

In all populations, the variance values of the 7-day total agglutinin responses are somewhat smaller than those presented in Table I. However, the results of the variance analysis are comparable, indicating that a similar gene interaction regulates both the 7-day and the 14-day responses.

The 7-day response of H mice mainly consists of 2-ME-R agglutinins which accounts for 96% of the total agglutinins. On the contrary, in the L line, only 60% of the 7 day agglutinins are 2-ME-R. Nevertheless, the immunogenetic characteristics of the total and 2-ME-R agglutinin responses are comparable.

The 14-day post-immunization responses consist almost entirely of 2-ME-R Ab in both H and L lines, and in the progeny of their various crosses. These data and the results of the variance analysis are similar to those reported in Table I (data not shown).

Life span. Females display a slightly longer mean life span than males, but the difference is not significant in all populations, which led us to pool together data from both sexes.

The cumulative mortality of the six distinct mouse populations are shown in Figure 1, which also indicates the median life span.

In each population, the mortality distribution conforms closely to the theoretical Weibull's curve, represented by the continuous line. The paired comparison of the mortality curves demonstrates statistically significant differences ($p < 0.02$) between H and L, F_1, and H, F_1, and L, F_1, and BcH, F_1, and BcL, BcH, and BcL. The difference between F_1 and F_2 is lower ($p = 0.1$). The mean life span values for the total population, for the first 10% dead as well as for the last lived 30%, 20%, 10%, 5%, and for the longest lived male and female are reported in Table III. The individual distribution of life span in the total populations is in acceptable agreement with the theoretical normal distribution curve, as the median and the mean life span are concordant in each population (Fig. 1, Table III). Nevertheless, the SD keeps correlated to the mean.

The mean age to death of the total population is a crude parameter for longevity, since it is largely affected by environmental factors related to disease incidence. Previous studies in selections I and II (29, 30) demonstrated that the shortened life span of L lines is partially due to a higher tumor incidence. The results of the present study confirm the impact of the specific disease incidence on life span (see Fig. 4). It is usually considered, though, that the life span of the small percentage of longest lived survivors is chiefly determined by the intrinsic genetic factors that regulate longevity throughout the physiological aging process (12, 41).

The longevity of H mice largely exceeds that of L mice for the total population, and all its percentages as reported in Table III. A marked difference also discriminates BcH from BcL populations ($p < 0.001$), except for the first 10% dead. This early mortality is very largely

TABLE II

Seven-day total and 2-ME-R SRBC agglutinin response in H and L responder line and in the progeny of interline crosses bred at ENEA, Casaccia, Rome, Italy[a]

Mice	No. of Mice	Total Agglutinins $\bar{X} \pm SD$	Variance (V)	2-ME-Resistant Agglutinins $\bar{X} \pm SD$	Variance (V)
H	134	11.4 ± 0.8	0.6	10.9 ± 0.8	0.6
L	123	5.2 ± 1.0	1.0	3.3 ± 1.0	1.0
F_1	153	9.4 ± 0.7	0.5	8.9 ± 0.7	0.5
F_2	192	9.8 ± 1.2	1.4	8.8 ± 1.4	2.0
BcH	174	10.8 ± 0.9	0.8	10.1 ± 1.1	1.2
BcL	102	7.5 ± 1.4	2.0	5.7 ± 2.0	4.0
		$a = 3.1$ $d = 1.1$	$d/a = 0.35$	$a = 3.8$ $d = 1.8$	$d/a = 0.47$
		$VE = 0.7 \pm 0.17$		$VE = 0.7 \pm 0.17$	
		$VG\ F_2 = 50\%$	VG Bc = 50%	$VG\ F_2 = 65\%$	VG Bc = 73%
		$VA\ F_2 = 0.66 \pm 0.4$	VA Bc = 1.25 ± 0.4	$VA\ F_2 = 1.17 \pm 0.7$	VA Bc = 3.11 ± 0.7
		$VD\ F_2 = 0.04$	VD Bc = 0.07	$VD\ F_2 = 0.13$	VD Bc = 0.35
		$n = 5.6 - 10.4$	$n = 3.3 - 4.6$	$n = 4.7 - 8.8$	$n = 2.1 - 2.6$
		$\bar{X}n = 5.8 \pm 2.5$		$\bar{X}n = 4.4 \pm 2.6$	

[a] n = n ranges. V BcH + V BcL = V Bc. For VE, VA, and n values: ± = SE. See text for other symbols.

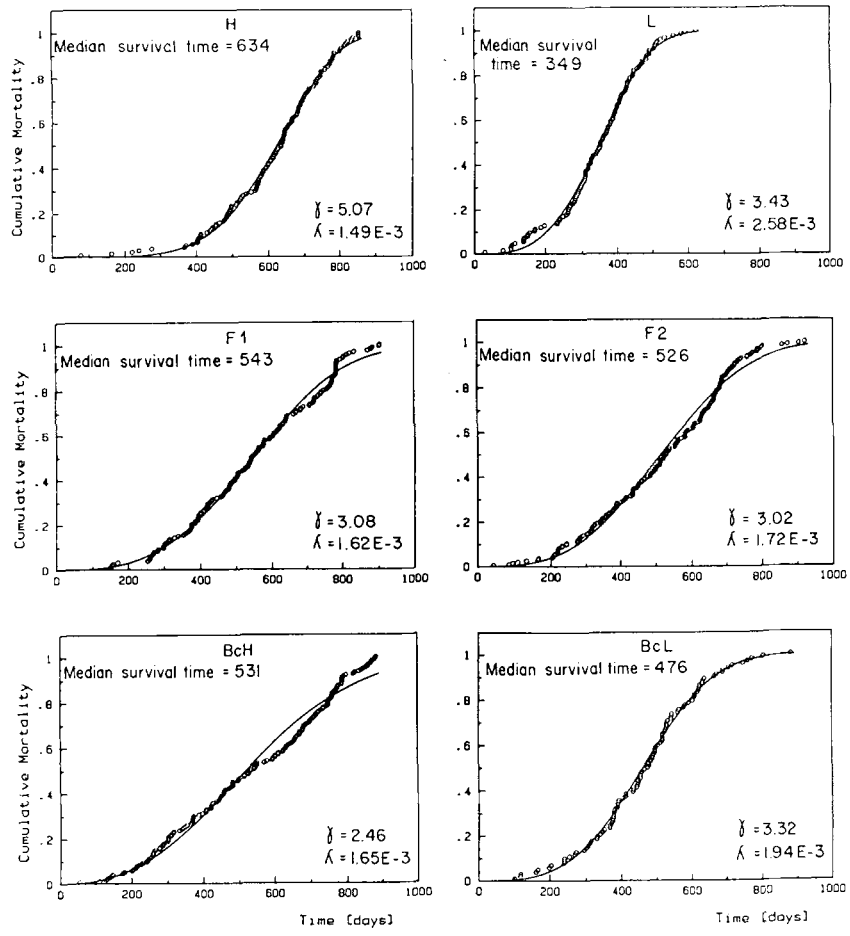

Figure 1. Cumulative mortality and median survival time in the various populations: H, L, (H × L)F_1, (F_1 × F_1)F_2, (F_1 × H)BcH, and (F_1 × L)BcL. Curves fitted as described in *Materials and Methods*.

TABLE III

Life span of H, L lines, and interline crosses in selection II

Mice	Life Span (days)							
	Total Population		Percent of Population					
	No. of mice	Mean ± SD	First 10% dead Mean ± SD	Last 30% survivors Mean ± SD	Last 20% survivors Mean ± SD	Last 10% survivors Mean ± SD	Last 5% survivors Mean ± SD	Last 2 survivors Mean ♀ ♂
H	134	612 ± 148	408 ± 137	694 ± 88	752 ± 64	787 ± 45	822 ± 25	850
L	123	346 ± 110	155 ± 52	407 ± 87	444 ± 74	490 ± 61	522 ± 59	605
F_1	153	549 ± 186	297 ± 87	670 ± 90	747 ± 62	785 ± 64	864 ± 60	892
F_2	192	513 ± 183	243 ± 83	639 ± 100	681 ± 85	724 ± 87	773 ± 78	859
BcH	174	530 ± 215	244 ± 84	693 ± 101	748 ± 71	786 ± 64	853 ± 20	879
BcL	102	464 ± 158	239 ± 82	531 ± 106	608 ± 100	670 ± 96	745 ± 70	819
H − L		266 ($p < 0.001$)	253 ($p < 0.001$)	288 ($p < 0.001$)	308 ($p < 0.001$)	298 ($p < 0.001$)	300 ($p < 0.001$)	245
a		133	127	144	154	149	150	122.5
d		70	15	119	159	147	152	164.5
d/a		0.53	0.11	0.83	1.03	0.99	1.01	1.34

affected not only by genetic factors acting upon disease incidence, but also by environmental effects, as demonstrated by the fact that the SD is correlated to the mean in all populations, regardless of their genetic constitution. This effect, though impinging mainly on the first 10% mortality, is large enough to also alter the SD of the whole population. Aversely, life span in the last 30% or less survival percentages (20%, 10%, and 5%), which are scarcely affected by early disease-induced mortality, is essentially determined by genes acting on the intrinsic physiologic longevity. The SD of genetically heterogeneous interline segregants (F_2 and Bc) are in fact larger than those of the genetically homogeneous populations (H, L, and F_1). It is interesting to note that the dominance effect (d/a) varies according to the parameter used for measuring life span. A very small dominance effect is found for the first 10% mortality group, in which the F_1 hybrids' life span is nearly intermediate between that of H and L lines. In the total population, there is a clear-cut incomplete dominance effect of long life character. This effect is stronger still for the last 30% survivors. When it comes to the last 20% or smaller percentages (10% and 5%), a complete dominance of the long life character can be observed, the F_1 hybrids living as long as the H mice. And it is remarkable that for the last two F_1 survivors, there is a definite overdominance effect of the long life character. The complete dominance effect of the long life trait in the last 20% or less long-lived mouse percentages explains the larger SD values in BcL compared with BcH.

Because the last survivors' (30%, 20%, 10%, and 5%)

SD values are consistent with a polygenic regulation model, the variance partitioning was performed by using the individual data of the last 30%, 20%, and 10% survivors. The results in Table IV indicate that physiologic longevity, as assessed in the longest lived mice, is a character determined by the interaction of genetic and environmental effects. The genetic component of the variances in the two segregant populations (*VG* F_2 and *VG* Bc) increases as the percentage of the last survivors decreases. Approximately 50% of the last 10% survival variance is due to genetic factors, whereas this ratio decreases to 25% in the last 30% survivors. The interaction of 3 to 7 independent loci accounts for approximately 40% of the individual variability in segregant hybrids, the remnant being determined by the effects of the environment. The major part of the genetic variability of F_2 and Bc (*VG* F2 and *VG* Bc) is produced by an additive genetic effect (*VA*); the remaining part, due to the dominance effect (*VD*), accounts for approximately 45% of the *VA* values. Postulating that the additive fraction of the genetic F_2 variance is heritable, h_2 could then be calculated, and is entered into Table IV. The h_2 value, related as it is to the percentage of *VG*, is maximal when taking into account the smallest percentage (10%) of the population.

The data in Table IV are in keeping with the hypothesis that intrinsic physiological longevity is a polygenic character regulated by a small number of independent loci (4.5 ± 1.5), with an h_2 value of about 0.3, largely affected moreover by environmental effects (60%).

Interpopulation correlation between Ab responsiveness and life span. Figure 2 represents the least square linear regression between H, L, F_1, F_2, BcH and BcL populations' life span and total as well as 2-ME-S agglutinin responses measured 7 days post immunization. The values of the slopes and of the correlation coefficient (r) for total agglutinin responses on the 14th day, and for 2-ME-R agglutinins on the 7th and 14th days are also indicated.

Results clearly demonstrate a very significant positive correlation between longevity and total or 2-ME-R Ab responses 7 and 14 days post immunization. The 14-day responses almost exclusively consist of 2-ME-R Ab, whereas an appreciable production of 2-ME-S agglutinins occurs 7 days post immunization. It has to be stressed that the correlation between longevity and 2-ME-S response is negative.

With regard to 2-ME-R agglutinin responses, a 30-day increase in life span corresponds to a doubling in Ab level (1 $\log_2$). Seven days post immunization, the slope × 30 days of total responses is decreased to 0.72 $\log_2$ due to the concomitant negative correlation of 2-ME-S Ab (slope = −0.21).

In L mice, 2-ME-S Ab represent 40% of total agglutinins, whereas in the H line, this proportion is reduced to 2%. In the various interline crosses investigated, the level of total and 2-ME-R agglutinin responses is intermediate between H and L values, which is consistent with the similar degree of incomplete dominance effect of the two characters investigated, Ab responsiveness (d/a) = 0.35—Table II) and longevity (d/a) = 0.53—Table III).

Intrapopulation correlations between life span and Ab responsiveness. Due to the dominance effect of the two characters investigated, life span and Ab responsiveness, the largest intraline individual variance was produced in the BcL population (Tables II, III, and IV). Consequently, the BcL segregants provide the best opportunity to demonstrate a positive individual correlation between the above two characters. In Table V*A* are summarized the results of the individual correlation between life span and total 2-ME-R and 2-ME-S agglutinin responses measured 7 and 14 days post immunization in BcL segregants.

The most significant positive correlation is found between life span and the 7-day 2-ME-R Ab response, whereas a weaker, but significant negative correlation is observed for 2-ME-S agglutinins.

A positive correlation is also found for the 14-day responses mainly or exclusively consisting of 2-ME-R Ab, although their slopes and significance levels are lower. This correlation is not verified for the total 7-day agglutinin response, because of the opposite effect due to the negative correlation of 2-ME-S Ab which are produced in this post-immunization period.

Since the early (7-day) 2-ME-R Ab titer gives the strongest correlation between Ab responsiveness and life span, this immunological parameter was elected to investigate that correlation in the other populations of interline crosses, that is F_1, F_2, and BcH. The results of the calculations, summarized in Table V*B*, indicate a weak positive correlation between Ab responsiveness and life span, which is at the limit of statistical significance.

As previously indicated for the total population, the left-hand tail of the normal mortality frequency distribution curve is mainly affected by disease-induced mortality, while the right-hand tail is essentially related to the physiologic aging-induced mortality. The individual correlation between 7-day 2-ME-R and mortality established in the first 20% dead of the BcL population gives: slope × 30 days = 0.35, r = 33, p = 0.1, whereas that established on the last 20% mortality gives: slope × 30 days = 0.43, = r = 0.52, $p < 0.02$. These results point out

TABLE IV

Variance analysis in the 30% and smaller percentages of the longest lived mice[a]

Percent of Population	*VE* ± SE	*VG* F_2 (%)	*VG* Bc (%)	F_2					Bc				
				VP	*VA*	*VD*	*n*	h_2[b]	*VP*	*VA*	*VD*	*n*	h_2[c]
Last 30%	7,804 ±156	22	27	10,000	1,638	558	6	0.16	21,437	3,469	1,180	3	0.35
Last 20%	4,472 ±507	38	40	7,225	1,799	954	7	0.25	15,041	2,959	1,569	4	0.41
Last 10%	3,280 ±637	56	50	7,569	2,878	1,411	4	0.38	13,312	3,410	1,671	3	0.45

[a] *V* Bc = *V* BcH + *V* BcL.
[b] VA F_2/VP F_2.
[c] VA Bc/VP F_2.
$\bar{X}n = 4.5 \pm 1.5$.

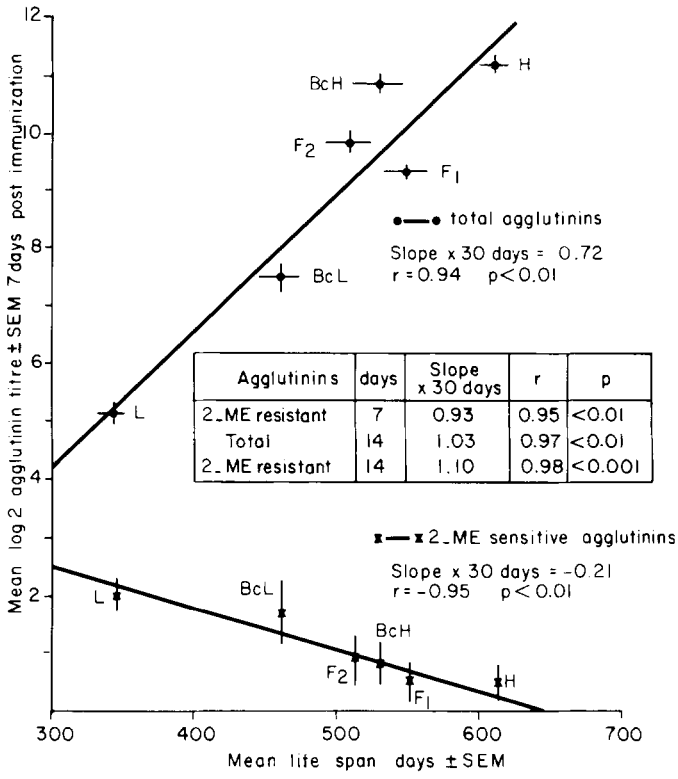

Figure 2. Least square linear regression between mean agglutinin responses and mean life span in H, L, F_1, F_2, BcH, and BcL (interpopulation correlation).

TABLE V

Individual intrapopulation correlation between life span and Ab responsiveness

A. Individual correlation[a] between life span and agglutinin responses in BcL segregants

Agglutinins	Day	Slope × 30 Days	Correlation Coefficient *r*	*p*
Total	7	0.049	0.15	0.2 (NS)
2-ME-R	7	0.150	0.33	<0.01
2-ME-S	7	−0.009	−0.20	<0.1
Total	14	0.087	0.20	0.05
2-ME-R	14	0.100	0.25	0.02

B. Individual correlation[a] between life span and 7-day 2-ME-R agglutinin responses in F_1 hybrids and in F_2 and BcH segregants

Mice	No. of Mice	Slope × 30 Days	Correlation Coefficient *r*	*p*
F_1	153	0.036	0.15	0.1
F_2	192	0.019	0.10	<0.2 (NS)
BcH	174	0.030	0.17	<0.05

[a] Least square linear regression: life span (x); Ab titer (y).

that Ab responsiveness is more closely correlated with the genetically programmed physiological longevity than with disease-induced life shortening.

The rather low r and slope values observed in all the above-reported intrapopulation correlations are essentially due to the large impact of environmental factors on both Ab responsiveness and life span (see *VE* in Tables II, III, and IV), which is amplified when individual correlation is being measured.

As we had in mind to reduce the impact of the environmental effects so as to get a finer evaluation of the correlation between genetic regulation of Ab responsiveness and life span, we calculated the mean and SD values of these two characters from data pooled on two different criteria.

First, the mean agglutinin response of mice within periods equivalent to 1/10 of the time separating the first and last dead mice, as shown in Figure 3*A*. Second, the mean life span calculated in groups of mice presenting the same agglutinin titer (1 $\log_2$ interval), as shown in Figure 3*B*.

Figure 3 shows the least square linear regression of 7-day 2-ME-R and 2-ME-S Ab responses in BcL population following the above two approaches. Slope and r values of the other populations (F_1, F_2, and BcH) are indicated as well.

The analysis presented in Figure 3 confirms the general meaning of the results shown in Table V. Although the experimental data fit much better the regression straight line, as shown by higher r values, the statistical significance of the results is not improved, because of the decreased number of paired data used to establish the correlation.

The results in Figure 3 give a definite confirmation of the opposite correlation between 2-ME-R vs 2-ME-S Ab and life span in the BcL segregant population. It may be calculated from Figure 3*B* that 2-ME-S agglutinins constitute 60% of total agglutinins in the shortest lived group of BcL, whereas they account for 19% in the longest lived group.

The tendency to a positive correlation between the 7 day 2-ME-R responses and longevity in the other interline crosses, F_1 and BcH, is also confirmed, as shown in Figure 3, *A* and *B*, though with lower significance and slope than in BcL mice.

The correlation between life span and Ab response obtained in pooled data (Fig. 3*A*) being consistent with that calculated in individual mice (Table V), we could investigate such correlation at Ab isotype level, using pooled sera. The 14 day sera were accordingly pooled by age-matched mouse groups, constituted as shown in Figure 3*A*. Ab titrations were made by indirect ELISA for either total IgG or distinct IgG isotypes.

These Ab titers, which largely depend on the affinity of the second Ab, cannot be compared to each other or to agglutinins. As could be expected, for all isotypes Ab titers in 14-day sera were high in H mice and undetectable in L mice (our unpublished data).

The data summarized in Table VI show, in the various populations of hybrid mice, the Ab titers' variability in the 10 age groups, as measured by the coefficient of variation (*CV*) and the parameters of least square linear regressions between $\bar{X}$ age (x) and $\bar{X}$ Ab titers (y). As expected, the *CV* is generally higher in segregants than in F_1 hybrids, except in BcH where the dominance effect of the high response lessens genetic variability.

The correlation obtained for the total IgG response is close to that found with 2-ME-R agglutinins in F_1, BcH and BcL populations. This correlation, which is not significant in F_2 for agglutinin titer (Table V and Fig. 3), is statistically significant for IgG ELISA titration, which is more accurate.

In all hybrids, the correlation concerning the distinct isotypes: IgG1, IgG2a, IgG2b, and IgG3, has a positive value (except for IgG1 in BcH). Highly significant r values are found in F_2 for all isotypes, whereas in BcH and BcL the degree of significance of r values varies according to the isotype.

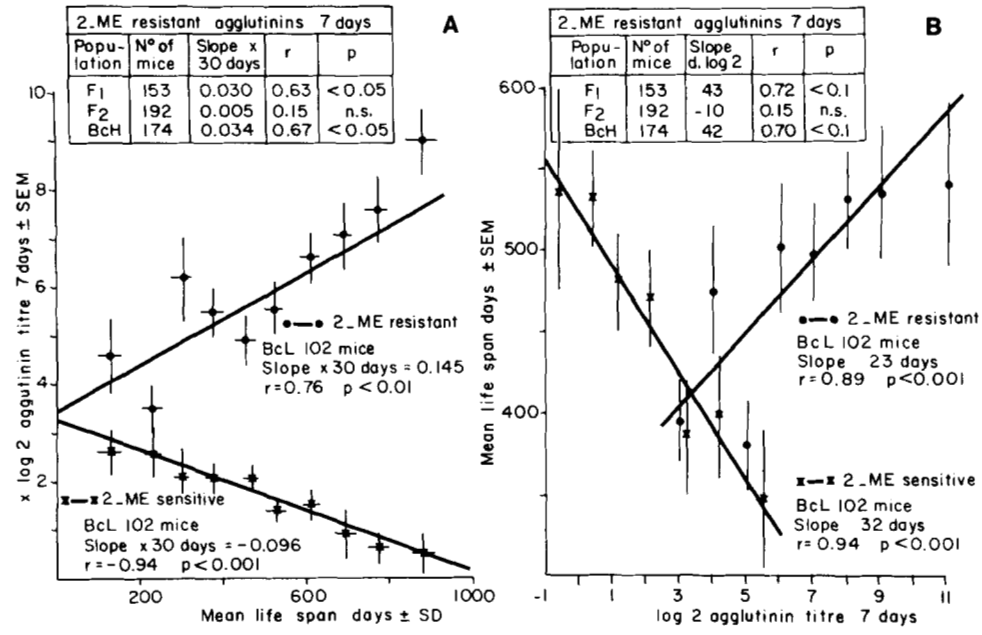

Figure 3. Least square linear regression between agglutinin responses and life span in F_1, F_2, BcH, and BcL mice. Data pooled in age matched groups (*A*) and agglutinin titer matched groups (*B*) (intrapopulation correlation).

The positive correlation is also generally significant in F_1 hybrids where, however, except for IgG1, the value of the regression slope is much lower than in segregant hybrids. As a whole, the results of IgG ELISA titrations confirm the positive correlation between 2-ME-R agglutinin and life span.

Pathology. A large spectrum of neoplastic and non-neoplastic diseases was observed at death time in the various populations investigated. A synopsis of pathologic data appears in Table VII.

Non-neoplastic diseases. Chronic nephritis was the major inflammatory disease observed in all populations investigated. Briefly, the kidneys were reduced in size and characterized by an irregular scarring of the cortex. Histologic observation revealed damage involving primary tubules, which were dilated and occasionally filled with colloid casts, a pattern referred to as tyroidization, associated with interstitial chronic inflammation and fibrosis. Various degrees of hyalinization were also present in the glomeruli, associated with mild periglomerular fibrosis. These kidney lesions were often accompanied with secondary deposition of amyloid substance in the liver and spleen, revealed by a positive Congo red stain and a green birefringence when observed by means of polarizing microscopy.

The age-adjusted incidence of chronic nephritis in H and L lines as in the progeny of their interline crosses is shown in Table VII, the significance of the differences between the various populations investigated in Table VIII. Results point out that the incidence of the renal disease correlates with the level of immune responsiveness. Age-adjusted incidences are significantly higher and more forward in L and BcL mice populations that present the lower Ab responses. A similar incidence in F_1 hybrids and in H line suggests a complete dominance of the "resistance" character against this inflammatory disease.

Mortality due to early incidence of chronic nephritis contributes to the shorter life span of the L and BcL populations (Fig. 1), affecting principally the first 10% of these populations (Table III, Fig. 4*A*).

Neoplastic diseases. Tumors of the lymphoreticular system were recorded according to the Pattengale classification (42, 43). Follicular center cell-lymphoma of B cell type was the most frequent among all lymphoid neoplasms, though a small number of lymphoblastic T cell type lymphomas was also observed (Table VII). The morphology of these neoplasms has already been described in previous articles (29, 30, 44).

The incidence data and the age-related death rate of malignant lymphomas are presented in Table VII and Figure 4*B*, respectively. The interpopulation significance of these incidence data is shown in Table VIII. All lymphoid neoplasms are more frequent in L responder mice and in the progeny of the various interline crosses than in the H responder mice. All interpopulation differences are statistically significant, except for the two Bc (Table VIII).

A large spectrum of benign and malignant solid tumors was also observed, mostly localized in the lungs, liver and mammary glands. Tumors at other sites, less frequent, are irregularly distributed among the various populations. Although the rough total incidence of all solid tumors is higher in H line (Table VII), when tumor incidence is being corrected by life span the L line appears to be more susceptible to tumor development than the H line. This difference is statistically significant at $p < 0.01$ (Table VIII).

Chronic nephritis and malignant lymphomas should be considered as the two main diseases accounting for the reduced life span of the two lower Ab responder populations: L and BcL, especially for the first 10% mortality (Table III).

DISCUSSION

The results in Tables I and II illustrate the remarkable magnitude and constancy, in two distinct environments, of the modification in Ab responsiveness produced by selective breeding. The extent of interline difference shown in Table I persists when mice are immunized at different periods of their life time (our unpublished observations). The analysis of the large mouse populations confirms our former findings on the 14-day total SRBC

TABLE VI

Intrapopulation correlation between SRBC IgG isotypes response and life span in the progeny of interline crosses between H and L responder lines[a]

	Total IgG			IgG1				IgG2a				IgG2b				IgG3			
	F_1	F_2	BcH	BcL	F_1	F_2	BcH	BcL[b]	F_1	F_2	BcH	BcL	F_1	F_2	BcH	BcL	F_1	F_2	BcH	BcL[b]
Variation coefficient, $(SD/\bar{X}) \times 100$	6.0	12.7	3.8	12.1	7.26	7.20	4.13	10.73	4.6	9.5	6.6	10.0	3.0	5.4	2.9	7.9	6.7	14.3	5.8	7.8
Correlation coefficient, r	0.68	0.72	0.51	0.80	0.87	0.69	−0.34	0.38	0.63	0.80	0.73	0.37	0.20	0.87	0.70	0.51	0.77	0.90	0.76	0.58
p	<0.05	<0.02	<0.2	<0.01	<0.001	<0.02	NS	NS	<0.05	<0.01	<0.02	NS	NS	<0.001	<0.02	<0.2	<0.01	<0.001	<0.01	<0.1
Slope × 30 days	0.044	0.082	0.022	0.094	0.056	0.046	−0.015	.033	0.025	0.060	0.043	0.023	0.005	0.037	0.017	0.031	0.040	0.093	0.041	0.057

[a] ELISA Ab titration made on pooled sera of mice included in 10 age groups, as in Figure 3A.
[b] Only female values included, values in males being too low.

agglutinin response (28), which demonstrated the polygenic regulation of this character, with a large impact of environmental effects (about 45%) and an incomplete dominance of high response ($d/a = 0.5$). The number of independent loci involved, previously estimated 2 to 8, is at present more finely determined as 5 to 8. Similar values of these immunogenetic parameters are found for the 7-day total and 2-ME-R agglutinin responses. This is in agreement with our results demonstrating a common global genetic regulation of all isotypes of both normal serum Ig (45) and specific Ab responses after immunization (our unpublished data).

All parameters used for measuring life span (Fig. 1 and Table III) clearly demonstrate a marked and constant superiority of H over L line ($p < 0.001$). This interline difference is similar (250 to 300 days) for all measurements (253 days for the first 10% dead and 245 days for the last two survivors). The genetic constitution of the two lines does then account for the early mortality, together with nephritis and lymphoma, and also for the later mortality due to physiologic aging. The impact of environmental factors is larger, though, in the 10% first dead mice than in the other population fractions, since in the distinct populations the SD values vary with the mean values rather than according to the genetic constitution. Conversely, in the 30% or smaller percentages of last survivors, the variance values are, as expected, larger in genetically heterogeneous F_2 and BcL segregants than in genetically homogeneous H, L, and F_1 populations. The results of variance analysis in the 30%, 20%, and 10% last survivors (Tables III and IV) clearly show that the dominance effect (d/a), the genetic variance (VG) and h_2 increase steadily as decreases the percentage of survivors.

Results from the 10% last survivors would thus be the most reliable evaluation of the genetic parameters regulating life span, in spite of the large error imputable to small sample size. As a matter of fact, the number of independent loci controlling life span is similar in the three population percentages considered. The data are consistent with the hypothesis that the difference in physiologic life span between H and L lines is regulated by the additive interaction of a small number of independent loci, and is largely affected by environmental effects ($VE = 60\%$). The large impact of environmental factors on the early death rate does not seem to be related to different sets of endemic viruses, since both H and L colonies and their hybrids were bred in the same room. The viruses' specific serology of the two lines shows occasional borderline increase of Ab threshold limited to H line mice. These mice have a higher level of natural Ab against several non pathogenic immunogens, such as heterologous E and proteins. Moreover, similar results on interline incidence of malignant lymphoma have been observed in two distinct mouse units (Institut Curie, Paris, and ENEA, Rome).

Significant differences in life span between some inbred strains of mice have been reported. At least 4 independent loci linked with coat color (chromosome 4), MHC (chromosome 17), sex, and Ah locus have been shown to influence the life span of inbred strains (8, 25, 46). The h_2 value for life span, as measured in the present study ($h_2 = 0.16$ to 0.45), is compatible with the estimate of genetic variability for life span in inbred strains, as

TABLE VII

Neoplastic and nonneoplastic diseases at spontaneous death in H or L responder mice and in the progeny of interline crosses

Specification	Tumor Type	H	L	F_1	F_2	BcH	BcL
No. of autopsied mice		131	119	153	191	174	102
Inflammatory diseases							
Chronic nephrytis		24	52	16	23	11	40
Percent incidence[a]		18 (18)	43 (70)	11 (12)	12 (19)	6 (9)	39 (52)
Lymphoid neoplasms							
FCC lymphoma[b]		18	24	66	45	66	27
Percent incidence[a]		14 (14)	20 (50)	43 (42)	24 (28)	38 (38)	27 (37)
Lymphoblastic lymphoma[c]			18	2	10	4	5
Percent incidence[a]			15 (27)	1 (2)	5 (6)	2 (3)	5 (5)
Total		18	42	68	55	70	32
Percent incidence[a]		14 (14)	35 (62)	48 (45)	29 (32)	40 (40)	31 (40)
Myeloid leukemia				5	1		
Solid tumors							
Lung	Alveolar adenoma	9		6	6	5	3
	Alveolar adenocarcinoma	6		14	9	11	8
Liver	Hepatocellular adenoma	4	4	5	5	4	
	Hepatocellular adenocarcinoma			18	14	7	5
GI tract	Adenocarcinoma	1					
Adrenal	Cortical adenoma	2					
Kidney	Adenoma				1	1	
	Adenocarcinoma					1	
Bladder	Carcinoma		1				
Skin	Squamous cell carcinoma	7	5	3	4	3	1
Soft tissues	Fibrosarcoma	1	1	6	7	7	
	Rabdomyosarcoma	3					
Vascular system	Malignant hemangioendothelioma	3	1		4	1	
Bone	Osteogenic sarcoma	3		1	4		
Salivary gland	Adenocarcinoma				1	1	
Utherus	Leiomyofibroma			2	1	2	
	Leiomyosarcoma				1		
	Schwannoma			1			1
Ovary	Tubular adenoma	1		1	2		
	Luteoma				1		1
	Teratocarcinoma				1		
Mammary gland	Adenoacanthoma			5	2	2	
	Adenocarcinoma	2	3	10	3	6	3
Total		42	15	73	67	51	22
Percent incidence[a]		27 (27)	12 (45)	39 (38)	29 (38)	26 (30)	18 (36)

[a] Numbers in parentheses are age-adjusted incidences (see text for details).
[b] Malignant lymphoma, follicular center cell (FCC) type.
[c] Lymphoblastic lymphoma, T cell-type.

TABLE VIII

χ^2 probability for heterogeneity of age-adjusted incidence of diseases in H or L responder mice, and in the progeny of interline crosses[a]

Line Comparison	FCC Lymphoma	T-cell Lymphoma	Total Lymphomas	Solid Tumors	Chronic Nephritis
H vs L	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$
F_1 vs H	$p < 0.001$	NS	$p < 0.001$	$p < 0.05$	NS
F_1 vs L	NS[a]	$p < 0.001$	$p < 0.01$	NS	$p < 0.001$
F_1 vs F_2	$p < 0.01$	$p = 0.06$	$p < 0.025$	NS	$p = 0.1$
BcH vs BcL	NS	NS	NS	NS	$p < 0.001$

[a] $p > 0.1$ = NS.

determined by the ratio between intraline and interline variances (0.20 to 0.37) (9, 11). These inbred strains, though, display but very small and irregular differences in Ab responsiveness to natural polydeterminant immunogens of distinct specificity. It seems then unlikely that the same genes can operate in inbred strains and in our H and L Ab responder lines.

The major purpose of the present study has been the demonstration of a correlation between Ab responsiveness and life span, correlation suggested by the similar modification of life span observed in three selective breedings: selections I and II, started from distinct foundation populations, and selection GS (47), characterized by an amplified interline difference in immunoresponsiveness. In this GS selection, the mean life span was 516 ± 162 days in H line, versus 319 ± 108 days in L line (our unpublished data). These concordant observations rule out the chance for a similar random drift fixation, in the three high or low lines, of genes affecting life span unlinked to those involved in immunoregulation.

The results of interpopulation correlation reported in Figure 2 are also compatible with the hypothesis of a causal relationship between immune responsiveness and life span. The conclusive results, however, are those obtained from intrapopulation correlation between the two characters in the interline segregant hybrids (Fig. 3, Tables V and VI). Highly significant positive correlation was demonstrated between life span and 2-ME-R agglutinin production in BcL population. The positive correlation was confirmed by ELISA for total IgG and for distinct IgG isotypes in BcL as well as in F_2 segregants.

A weak positive correlation between Ab response and life span was also constantly observed in the genetically homogeneous F_1 hybrid population. This finding clearly indicates that the individual life span is determined by the level of immune responsiveness, whatever be its origin (genetic or environmental).

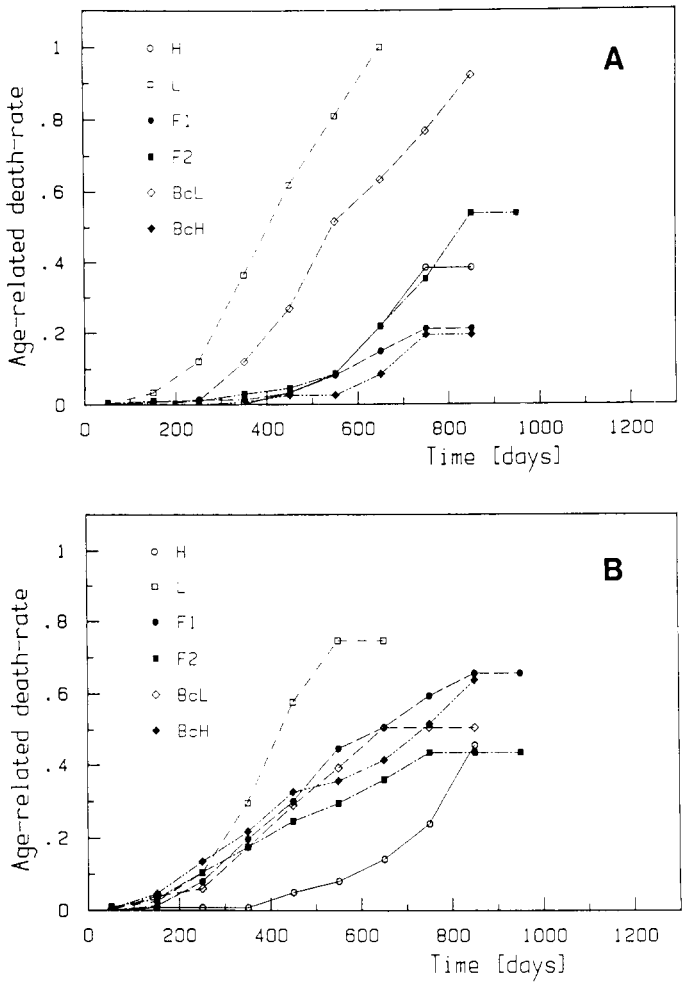

Figure 4. Cumulative age-specific death rates for nephropathies (*A*) and malignant lymphomas (*B*) in H, L, F_1, F_2, BcH, and BcL populations.

An unexpected finding was the lower, but significant, negative correlation between 2-ME-S agglutinin response and life span, as demonstrated in both inter and intrapopulation studies. The seeming detrimental effect of 2-ME-S Ab on life span may result from genetic modification of the regulatory mechanisms activating the switch from IgM to IgG during the immune response. Impairment of the ability to switch from low affinity IgM to high affinity cytophilic IgG antibodies is likely to affect life span negatively.

The intrapopulation positive correlation between Ab responsiveness and life span in segregant interline hybrids demonstrates that these two characters are controlled by the interaction of either one set of pleiotropic genes, or two sets of closely linked loci. According to the first hypothesis, the apparent pleiotropic gene effect would, in fact, result from a causal relationship between immune responsiveness and life span. The detrimental effect of low response on life span is more obvious than the beneficial effect on it of high response. This results from the asymmetric effect of the selective breeding on both Ab response and life span. When compared to the foundation population (28) or to F_2 hybrids (Table III), the decrease of these two characters in the L line is larger than their increase in the H line.

The conclusion that Ab responsiveness and life span have a common polygenic regulation is not backed up by the results of selection III, according to which the H and L Ab responder lines do not differ in either life span or tumor incidence (30). But what looks at first like a discrepancy happens, in fact, to shed some light upon how to identify the immunologic mechanisms influencing life span. In Selections I and II, based on primary Ab response, the difference between H and L responder lines is chiefly due to genetic modifications in macrophage Ag metabolism and presentation (2, 48). This interline difference therefore involves humoral and T cell mediated responses (49). Selection III, on the contrary, was based on secondary response, and no detectable difference in accessory cell activity between H and L Ab responder lines could ever be demonstrated, which suggests that it is only the genetic regulation of humoral responsiveness that is being affected (50, 51). The concomitance of high primary Ab responsiveness, longevity and low tumor incidence, as demonstrated in the present study, might reflect an increased expression of T cell-mediated immunity. The major role of T cells in genetic resistance against spontaneous tumors is also brought forth by the observation that a line of mice selected for low T cell response to T mitogens displays a higher tumor incidence than the high T cell responder line (44, 52).

It should be stressed that, although selection II was carried out for Ab response to SRBC, its effect is general upon responsiveness to many unrelated immunogens (1). The high incidence of malignant lymphoma in L line is consistent with the clinical observation of frequent occurrence of lymphoma in transplanted humans submitted to drug-induced general immunosuppression. This consideration substantiates the above-formulated hypothesis on the protective effect of an efficient immune system against neoplasia.

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