Mechanisms of Ageing and Development, 36 (1986) 79–93
Elsevier Scientific Publishers Ireland Ltd.

INTERACTION OF *H-2* GENOTYPE AND BASAL SERUM IMMUNOGLOBULIN A LEVEL INFLUENCES LONGEVITY

DIANA M. POPP, J.A. OTTEN and R.A. POPP
Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 (U.S.A.)

(Received March 7th, 1986)
(Revision received May 16th, 1986)

SUMMARY

The congenic pair of mice, C57BL/10 (B10) and C57BL/10.F (B10.F), differ at the *H-2* locus and have mean ages at death of 706 and 456 days, respectively. B10.F also has reduced basal serum IgA levels compared with B10, 63 and 256 mg/dl, respectively. Controlled matings between the two strains of mice were used to identify genetic factors that govern longevity. F_2 and backcross progeny from reciprocal F_1 hybrids were classified for *H-2* genotype and serum IgA levels and allowed to live out their lifespan. F_2 and backcross progeny homozygous for the *H-2* allele of B10.F had a mean age at death (602 days) significantly reduced from that of progeny homozygous for the *H-2* allele of B10 (689 days). However, the greatest reduction of lifespan occurred among progeny of the (B10.F × B10)F_1 mothers, 693 compared with 540 days. The strain of the maternal parent also has been shown to affect the segregation of IgA phenotypes. An increased incidence of low IgA phenotype associated with *H-2* genotype was observed among progeny of (B10.F × B10)F_1 mothers. Survival curves demonstrated a relationship between low serum IgA levels and shortened lifespan and no maternal effect was observed. The basis of the shortened lifespan among progeny of F_1 hybrids in which the maternal parent was B10.F was the increased incidence of offspring with low IgA phenotypes. The apparent association of *H-2* and shortened lifespan also was because the low IgA phenotype was more frequent among progeny that carried the *H-2* allele of the B10.F strain. The B10.F mice spontaneously shed an endogenous ecotropic retrovirus which may be responsible for the maternal effect on immunoglobulin levels and lifespan.

Key words: Lifespan; Genetics; Immunoglobulin; Retrovirus; Maternal Effect

---

This research was sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.

0047-6374/86/$03.50
Printed and Published in Ireland

INTRODUCTION

The members of a congenic pair of mice, C57BL/10 (B10) and C57BL/10.F-*H-2* (B10.F), have been reported to have significantly different life spans [1]. This pair has also been shown to differ in basal immunoglobulin A (IgA) levels [2]. Using F_2 and backcross progeny from reciprocal F_1 hybrids of B10 and B10.F, both life span [3] and serum IgA levels [4] have been shown to be influenced by an unidentified maternal effect. Genetic tests also showed that lifespan could be correlated with *H-2* genotype, i.e. progeny homozygous for the $H\text{-}2^n$ allele of the short-lived strain (B10.F) had a mean age at death significantly reduced from that of progeny homozygous for the $H\text{-}2^b$ allele of B10 [3]. It was also noted that *H-2* genotype and maternal parentage were synergistic in life span reduction; the $H\text{-}2^{n/n}$ progeny from the (B10.F × B10)F_1 survived less well than the $H\text{-}2^{n/n}$ progeny from the (B10 × B10.F)F_1 mother. The basis for the observed maternal effect on life span is examined in this report and evidence is presented showing that low IgA levels in association with the $H\text{-}2^n$ allele shortens longevity. The results of cell transfer and foster nursing experiments that were designed to examine the mechanism of the maternal effect are also reported. Data are presented that suggest that virus reintegration in B10.F somatic cell DNA may account for the influence of the maternal parent on the inheritance of IgA phenotypes observed in progeny of B10.F mice.

MATERIALS AND METHODS

Mice

The origin of the B10 and B10.F congenic pair used in this study is described in a previous report [3]. Backcross and F_2 progeny were produced from reciprocal hybrid females derived from strains B10 and B10.F to study the influence of *H-2* genotype, IgA phenotype and maternal parent on longevity. Each offspring was serotyped for *H-2* genotype, classified according to IgA level, and the date of natural death was recorded. Parental strains and all progeny were maintained in the same room and under equivalent environmental conditions.

Serology

The hemagglutination method of Gorer and Mikulska [5] was used to determine the *H-2* serotypes of the offspring. The antiserum used to detect the $H\text{-}2^n$ haplotype of the B10.F parent was made in (B10.A × A.CA)F_1 against B10.Y tissue (designated C-16) and was obtained from the Transplantation Immunology Branch of the National Institutes of Health. The antiserum used to detect the $H\text{-}2^b$ haplotype of the B10 parent was made in C3H mice against RFM tissues.

Immunoglobulin (IgA) quantitation

The procedures for serum IgA quantitation are described in a previous publication [4]. In brief, the Mancini radial immunodiffusion method [6] was used, employing immuno-

diffusion plates obtained from Meloy Laboratories. All offspring were 4 months or older when basal serum IgA levels were determined.

Cellular studies

Spleen cells were suspended in Eagle's MEM without bicarbonate [7]. Spleens were teased apart and single cell suspensions were prepared by aspirating through a 21-gauge needle. The cell concentrations were adjusted to 75×10^6/ml and 0.2 ml were injected intraperitoneally into suckling offspring. A B10.F spleen suspension was centrifuged at 30 000 *g* for 1 h to yield a cell-free preparation. The supernatant was injected into 15-day-old B10 neonates. These recipients received the same spleen-equivalent dose as the recipients of the whole cell inoculum.

Bone marrow was flushed from the femurs of donor mice and suspended in Eagle's MEM (without bicarbonate). Lethally irradiated (800 R) recipients received injections of 15×10^6 cells via the tail vein. Litters selected to be foster nursed were removed from their mothers at birth and transferred to post-partum females whose own offspring had been removed prior to suckling.

The females that provided the blastocysts were mated and examined daily for copulation plugs. At 3.5 days post-plug the uterus was removed and flushed with Brinster's solution (Gibco Laboratories). The blastocysts collected in this manner were introduced into surrogate mothers by injection into the surgically exposed uterus. Six to ten blastocysts were introduced at a time. The appropriate hormonal state for blastocyst implantation in the recipient female was assured by pairing these females with vasectomized males and examining for copulation plugs.

Virus assays

Spleens from young (4-month-old) B10 and B10.F mice were aseptically removed and frozen (−70°C) in 1.0 ml of phosphate buffered saline. The spleens were thawed, homogenized in a glass homogenizer, and the supernatant filtered through a 0.45 μm filter. The filtered supernatant was assayed for virus on SC1 cells [8] by fluorescent antibody and XC assays [9,10]. At 48 h post-infection, the medium was removed from the SC1 cells and saved as small virus pools. The virus pools were used to infect A31 [11], NIH [12] and SC1 cells, and 5 days later the XC assay was performed as previously described [9].

Southern blot analysis

DNA was prepared from the liver, spleen, and pooled peripheral and mesenteric lymph nodes of B10 and B10.F mice. The DNA was digested by *PvuII* and electrophoresed in 1% agarose, transferred to nitrocellulose paper, and hybridized with a 0.4 kb probe specific for the *env* gene of ecotropic AKR MuLV [13].

RESULTS

An analysis of F_2 and backcross progeny of reciprocal F_1 hybrids from B10 and B10.F

TABLE I

THE MEAN AGE AT DEATH ± S.E.M. OF *H-2* SEGREGANTS IN F_2 AND BC POPULATIONS FROM RECIPROCAL F_1 HYBRID FEMALES [3]

No. of animals in parentheses.

H-2 genotype	*Maternal parent*	
	(B10 × B10.F)F₁	*(B10.F × B10)F₁*
b/b	687 ± 18 (58)	693 ± 23 (30)
n/n[a]	627 ± 15 (89)	540 ± 19 (35)

[a]Historically, the *H-2* haplotype of B10.F was defined as $H\text{-}2^n$. Subsequent studies showed serological similarity with $H\text{-}2^p$ although skin grafts between $H\text{-}2^p$ and $H\text{-}2^n$ congenic lines were rejected in one direction. Thus, the *H-2* haplotype of B10.F has been designated a minor variant of $H\text{-}2^p$ [19]. We will use $H\text{-}2^n$ in this report to be consistent with the series of papers that have been published in the course of these studies.

mice showed that the maternal parent influences the segregation of serum IgA phenotypes [14]. Survival curves of these progeny sorted on the basis of their *H-2* types showed that the class with the $H\text{-}2^{n/n}$ genotype had a shortened life span and a maternal effect also influenced the severity of life shortening [3]. These data are summarized in Table I. For the purposes of this report, these data have been re-examined in Table II to show the distribution of IgA phenotypes among the progeny of each mating and the distribution of IgA phenotypes according to *H-2* genotype. Data in Table II show that a larger proportion of the F_2 and B10.F backcross offspring from (B10.F × B10)F_1 females

TABLE II

THE DISTRIBUTION OF SERUM IgA PHENOTYPES (%) AMONG F_2 AND BACKCROSS (BC) PROGENY FROM RECIPROCAL F_1 HYBRID FEMALES

No. of animals in parentheses.

H-2 Genotype:	*All*		*b/b*		*n/b*		*n/n*	
IgA Phenotype[a]*:*	*High*	*Low*	*High*	*Low*	*High*	*Low*	*High*	*Low*
(B10 × B10.F)F_1								
F_2 (193)	72	28	82	18	66	34	75	25
B10 BC (41)	73	27	77	23	68	32	–	–
B10.F BC (41)	84	16	–	–	92	8	77	23
(B10.F × B10)F_1								
F_2 (73)	49	51	93	7	49	51	16	84
B10 BC (31)	74	26	94	6	53	47	–	–
B10.F BC (32)	25	75	–	–	50	50	0	100

[a]IgA high > 100 mg/dl; IgA low < 100 mg/dl (1).

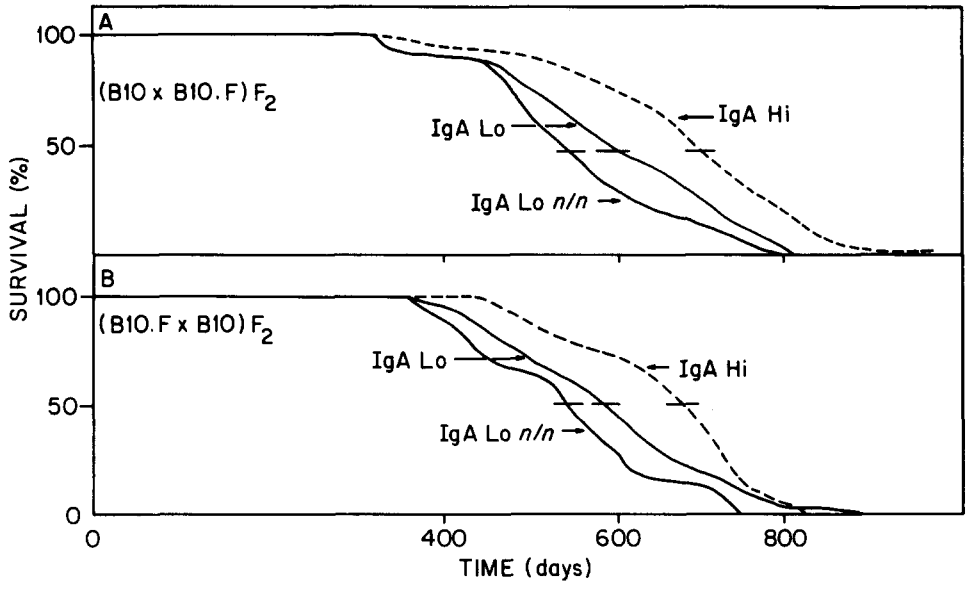

Fig. 1. Survival curves of F_2 progeny of reciprocal F_1 hybrids classified according to IgA phenotype. Lo = Low level; Hi = high level; n/n = *H-2*^{n} homozygote.

have the low IgA phenotype than progeny from similar matings using the reciprocal F_1 hybrid female. The distribution of IgA-low- and IgA-high-phenotypes is similar for each of the *H-2* segregant groups born by (B10 X B10.F)F_1 females. However, among the progeny of (B10.F X B10)F_1 females the distribution of IgA phenotype is different for each of the *H-2* segregant groups and a greater proportion of *H-2*$^{n/n}$ and *H-2*$^{n/b}$ segregants have low IgA levels compared with similar progeny from the reciprocal F_1 parent.

Since the populations that show reduced lifespans have an increased incidence of low-IgA-phenotypes, survival curves of (B10 X B10.F)F_2 and (B10.F X B10)F_2 selected by IgA phenotype were constructed (Fig. 1). The progeny that have high serum IgA levels survive better than those that have low IgA levels in both of the reciprocal F_2 populations. The 50% lifespan of the high or low IgA groups is not influenced by the maternal parent; thus, there is no maternal effect imposed upon the survival data when expressed as a function of IgA levels. When the 50% lifespan of IgA-high- and IgA-low-progeny are compared among the reciprocal BC progeny, there is no maternal effect evident (Table III). The reduced survival associates with the IgA low phenotype, and the relative survivals of high and low groups are similar whether descended from (B10 X B10.F)F_1 or (B10.F X B10)F_1 female parents (Table III), except for the (B10 X B10.F)F_1 backcross to B10 where the survivals of the IgA low and high groups are equivalent. (See Discussion Section.) In addition, the survival of the *H-2*$^{n/n}$ segregants of the IgA low populations from the reciprocal F_2 matings is reduced equally (Fig. 1) and there is no evidence of a maternal

TABLE III

THE 50% LIFESPAN (DAYS) OF F_2 AND BACKROSS MICE FROM RECIPROCAL F_1 HYBRIDS DERIVED FROM STRAINS B10 AND B10.F CLASSIFIED ACCORDING TO IgA PHENOTYPE; 50% SURVIVAL WAS DETERMINED FROM SURVIVAL CURVES

(B10 × B10.F)F₂			*(B10.F × B10)F₂*		
IgA phenotype	*No. mice*	*50% lifespan*	*IgA phenotype*	*No. mice*	*50% lifespan*
High	133	680	High	34	670
Low	53	590	Low	37	580
(B10 × B10.F)F₁ × B10			*(B10.F × B10)F₁ × B10*		
High	27	695	High	21	710
Low	11	695	Low	8	600
(B10 × B10.F)F₁ × B10.F			*(B10.F × B10)F₁ × B10.F*		
High	44	650	High	8	710
Low	9	480	Low	24	560

effect. Thus, data on the lifespan of reciprocal progeny were pooled to analyze the survival of groups selected according to their IgA level and *H-2* genotype.

The mean age at death of *H-2* segregants sorted according to their IgA phenotype are given in Table IV. Among the progeny that have high serum IgA levels, the survival rate

TABLE IV

THE MEAN AGE AT DEATH (DAYS) ± S.E.M. OF F_2 AND BACKCROSS PROGENY OF RECIPROCAL F_1 HYBRIDS OF STRAINS B10 AND B10.F CLASSIFIED ACCORDING TO THEIR IgA PHENOTYPE AND *H-2* GENOTYPE

IgA: H-2 types	*No. mice*	*Mean age at death*	*P*
High IgA			
b/b	70	685 ± 17	–
n/b	130	688 ± 11	NS
n/n	67	651 ± 17	NS
Low IgA			
b/b	13	688 ± 27	NS
n/b	74	623 ± 16	<0.01
n/n	54	539 ± 16	<0.001

Statistical comparisons have been made with the high IgA, $H\text{-}2^{b/b}$ group taken as representative of the normal lifespan of strain B10. Student's *t*-test was used to determine significant differences ($P > 0.05$ = NS, not significantly different). Hewlet-Packard 65 Stat Pac 1-30A.

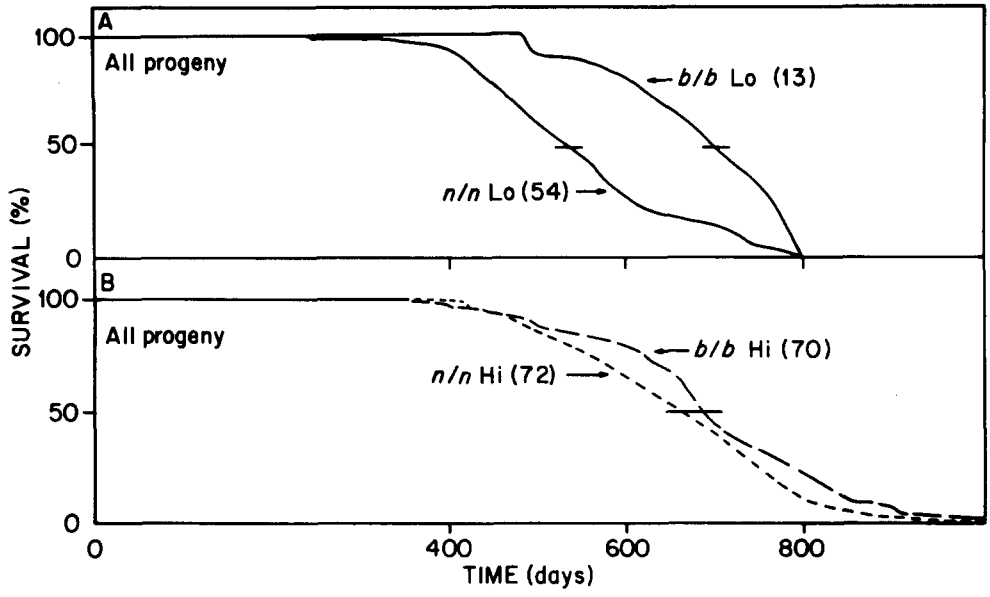

Fig. 2. Survival curves of homozygous *H-2* populations pooled from F_2 and backcross progeny of reciprocal F_1 hybrids, classified according to IgA phenotype. No. of animals in ( ). Lo = low level; Hi = high level; n/n = $H\text{-}2^n$ homozygote; b/b = $H\text{-}2^b$ homozygote.

is not different with respect to *H-2* genotype; the $H\text{-}2^{n/n}$ progeny have a slightly reduced mean age at death but the difference is not significant ($P > 0.05$). Among the progeny that have low serum IgA levels, the survival of the *H-2* segregants is different. The survival of $H\text{-}2^{b/b}$ progeny is comparable with that of progeny with high serum IgA levels, but progeny with low serum IgA levels *and* homozygous for the $H\text{-}2^n$ allele are severely compromised with respect to lifespan. Individuals that are heterozygous at *H-2* also have significantly reduced survival. Fig. 2 shows that $H\text{-}2^{n/n}$, high IgA and $H\text{-}2^{b/b}$, low IgA progeny have survival curves indistinguishable from that of $H\text{-}2^{b/b}$, high IgA progeny. Only the combination of $H\text{-}2^{n/n}$ and low serum IgA levels severely shortens lifespan. Survival of *H-2* heterozygotes that have low serum IgA levels is reduced but not as much as that of the $H\text{-}2^n$ homozygotes.

A foster-nursing study was done in attempts to understand the maternal effect on serum IgA levels. Newborn B10.F, (B10 X B10.F)F_1 and B10 mice were placed with post-partum females in the combinations shown in Table V. When B10.F neonates were foster nursed on B10 females, their adult IgA phenotypes were not altered. However, all B10 and (B10 X B10.F)F_1 offspring that had been foster nursed on B10.F mothers had reduced serum IgA levels that were similar to those of B10.F mice at 4 months of age. At 1 year, the IgA levels were still suppressed in the foster-nursed B10 mice and in 4 of the 6 foster-nursed (B10 X B10.F)F_1 mice.

To assess the possibility that maternally derived immunocompetent cells modulated the maturation of the humoral IgA response of immature mice, spleen cell transfers

TABLE V

BASAL SERUM IgA LEVELS OF 4-MONTH-OLD MICE THAT WERE FOSTER NURSED AT BIRTH

Offspring	*Foster mother*	*IgA (mg/dl)*
B10.F	B10	49, 65, 52, 57, 55, 74, 63
(B10 X B10.F)F_1	B10.F	63, 55, 100, 115, 100, 80
B10	B10.F	57, 57, 49, 50, 59
B10	B10.F	65, 65, 65

were made into 12-day-old neonates before IgA synthesis begins. Because B10 and B10.F differ at *H-2,* these cell transfers had to be done using F_1 hybrids. The data are presented in Table VI. In three experiments in which spleen cells from adult (B10.F X B10)F_1 donors (low serum levels) were injected into (B10 X B10.F)F_1 neonates (potentially high serum IgA levels), 12 of 12 surviving recipients had low serum IgA levels at 4 months. The serum IgA levels were still low at 1 year; 6 survivors at 12 months had suppressed serum IgA levels and the last survivor was still suppressed at 19 months (later data not given).

TABLE VI

BASAL SERUM IgA LEVELS OF 4 MONTH OLD (B10 X B10.F)F_1 MICE THAT RECEIVED SPLEEN CELL INJECTIONS AS NEONATES[a].

Donor	*IgA (mg/dl) 4 months*	*IgA (mg/dl) 12 months*
Experimentals		
(B10.F X B10)F_1 Adult spleen	59, 55, 47, 47, 47	D[b], 64, 44, 68, D
(B10.F X B10)F_1 Adult spleen	55, 55, 50, 55, 50	D, 77, 71, D, 55
(B10.F X B10)F_1 Adult apleen	65, 65	56, 56
Controls		
(B10 X B10.F)F_1 Adult spleen	75, 75	97, 157
(B10 X B10.F)F_1 Adult spleen	84, 71, 51, 56	200, 360, 77, 285
Experimental		
(B10.F X B10)F_1 17-day-old spleen	56, 56, 48, 41, 48	97, 68, 38, 77, D

[a]All neonate recipients were 12 days old except for the 17-day-old recipients of 17-day-old spleen.
[b]D, dead.

The controls, (B10 X B10.F)F_1 recipients of (B10 X B10.F)F_1 adult spleen, demonstrated the unexpected effect of adult isologous spleen to generally suppress (or delay) IgA expression. This effect was temporary; 4 of the 6 controls had achieved a high IgA serum level by 1 year (Table VI). To determine if the effect of injecting adult (B10.F X B10)F_1 spleen was due to the introduction of mature immunoregulatory cells (T suppressor cells) into a developing immune system or inherent in the genome of the B10.F maternal strain, spleen cells from 17-day-old (B10.F X B10)F_1 mice were injected into 17-day-old (B10 X B10.F)F_1 offspring. All 5 recipients had low serum IgA levels when tested at 4 months of age. These recipients were still suppressed at 1 year and one survivor was still suppressed at 18 months. Adult bone marrow injected into neonatal mice was also capable of modulating the IgA levels (data not given).

A line of mice was developed from two (B10 X B10.F)F_2 that had the *H-2*$^{b/b}$ genotype of B10 mice and the low serum IgA level of the B10.F strain. Spleen cells from these mice were used to show that similar modulation of serum IgA levels could be induced in B10 mice (Table VII). Spleen transfers into 8-day-old neonates modulated serum IgA levels in the B10 recipients and they were still suppressed at 1 year. Spleen cell transfers into 30-day-old weanlings modulated serum IgA levels but the modulation was temporary. Irradiation of the spleen cells did not abrogate the effect on IgA levels, and 4 of 6 recipients of the irradiated spleen cells were still modulated at 1 year.

The development of the *H-2*$^{b/b}$, IgA low line also permitted an experiment to assess the effect of bone marrow transplantation on serum IgA levels. The transfer of B10 bone marrow into lethally irradiated B10 adult recipients did not alter the normal serum IgA levels for 4 of the 5 B10 recipients (Table VIII). Reciprocal bone marrow transfers between B10 and B10.F mice resulted in death of the recipients due to graft versus host

TABLE VII

THE BASAL SERUM IgA LEVELS OF 4-MONTH-OLD B10 MICE THAT RECEIVED SPLEEN CELLS FROM DONORS THAT WERE *H-2*$^{b/b}$ AND HAD LOW SERUM IgA LEVELS (b/b LOW)

Donor	*Recipient*	*IgA (mg/dl)*	
		4 months	*12 months*
b/b Low	B10	72	D[a]
Adult spleen	8 day neonate	56, 56, 46, 51, 46	54, 47, 54, D, D
b/b Low	B10		
Adult spleen	30 day weanling	56, 48, 56, 52, 56	400, 138, 250, 123, 110
b/b Low	B10	77, 56	52, 84
Adult spleen 2163r X-ray[b]	12 day neonate	61, 90, 56, 41	32, 123, 137, 84

[a]D, dead.
[b]Irradiated *in vitro*.

TABLE VIII

BASAL SERUM IgA LEVELS OF RADIATION-INDUCED CHIMERIC MICE

Adult donor bone marrow	*Adult host*	*IgA (mg/dl)*
B10	B10	395, 490, 65, 650, 415
B10	B10.F	(gvh)[a]
B10.F	B10	(gvh)
b/b Low	B10	66, 78, 71, 61, 56, 61

[a]Lethal graft-versus-host disease.

disease. When the low IgA *H-2*$^{b/b}$ mice were used as bone marrow donors, the B10 recipients survived and expressed reduced levels of IgA in their serum.

Some of the IgA suppressed (B10 X B10.F)F_1 and B10 females were mated to produce progeny. The IgA suppressed (B10 X B10.F)F_1 females (Table VI) produced two F_2 litters, all F_2 progeny had low IgA levels; when these F_2 produced F_3 progeny, all F_3 progeny had serum IgA levels normal for B10 mice. The 2 B10 females that were suppressed by the injection of irradiated spleen (Table VII) produced 19 progeny, 17 of these were suppressed but their progeny had normal serum IgA levels.

Blastocyst transfer was done to examine the influence of the uterine environment on IgA levels. Three survivors were raised following the transfer of (B10.F X B10)F_1 blastocysts to a B10 surrogate mother. They were H-2 serotyped to establish that they developed from the transferred blastocysts. All 3 offspring had elevated IgA levels (180, 170 and 318 mg/dl), even though they would have had low IgA levels had they developed naturally in B10.F mothers.

The B10 neonate recipients of cell-free spleen supernatants were tested for IgA level at 4 and 7 months of age. At 4 months, 7 of the 9 recipients were suppressed and at 7 months 6 of the 9 were suppressed (data to be published). In addition, 1 female and 3 males showed signs of early greying.

Spleens from young B10 and B10.F mice were tested for virus expression. Table IX shows that the proteins detected by the immunofluorescence assay and the infectious virus subsequently detected by the XC plaque assay were present in the spleens from both strains of mice. The immunofluorescence assay was performed by placing supernatant from a spleen homogenate on SC1 cells followed by examination of the cells 6 days later. No enhancement of virus in the spleen cells was done prior to the immunofluorescent assay. The data shown in Table IX indicate that more virus was present in the B10.F spleen cells (94% positive cells) than in the B10 cells (20% positive cells). Comparable numbers of SC1 cells were treated with the supernatants and, in addition, the spleens used to prepare the supernatants were equivalent in weight. The SC1 cells multiplied at similar rates and, at the time of coverslip harvest, equivalent numbers of cells were counted. The additional virus load in the B10.F, as indicated by the immunofluorescent assay, is consistent with the suggestion that this strain of mouse, as opposed

TABLE IX

THE GROWTH OF VIRUS ISOLATES FROM B10 AND B10.F SPLENIC HOMOGENATES ON A31 (Fv-1^b), NIH/3T3 (Fv-1^n) AND SC1 (Fv-1$^-$) CELLS

Test cell	*Virus analysis*			
	FA[a]		*XC*[b]	
	B10	*B10.F*	*B10*	*B10.F*
SC1	20%	94%	10^{-5} (32)[d]	10^{-5} (76)
A31	ND[c]	ND	10^{-2} (5)	10^{-2} (60)
NIH/3T3	ND	ND	10^{-1} (18)	10^{-1} (32)

[a]Direct infection of SC1 cells. Coverslips harvested at 6 days.
[b]Infection of SC1, A31 and NIH/3T3 cells with small virus pools prepared in SC1 cells. XC at 5 days.
[c]Not done.
[d]Number of plaques at indicated dilution of virus pools are given in parentheses.

to the B10, carries with it an additional virus burden from birth that may be responsible for the observed maternal effect.

The tropism of the virus isolates from B10 and B10.F spleens was determined by infecting A31 (*Fv-1*^{b}) and NIH/3T3 (*Fv-1*^{n}) cells with the virus pools. In addition, SC1 (*Fv-1*$^-$) cells were infected to determine the titers of the virus contained in the individual virus pools. The data in Table IX show that both virus isolates demonstrated preferential growth on the A31 (*Fv-1*^{b}) cells as opposed to the NIH/3T3 (*Fv-1*^{n}) cells which indicates that both virus isolates are B-tropic. When compared with the titers in SC1 (*Fv-1*$^-$) cells, virus from the B10 and B10.F mice show an apparent restriction in both the N- and B-tropic mouse cells. These results show that the virus is present in both strains of mice and that the B10.F strain has a heavier virus burden from an early age than the B10 strain of mouse.

To explore the basis for the difference in viral shedding between the congenic pair, Southern blot analysis was carried out on B10 and B10.F DNA prepared from liver, spleen, and pooled mesenteric and peripheral lymph nodes. The 0.4 kb *env* specific probe used identifies the endogenous ecotropic locus (*Emv-2*) present in B10 mice as a 5.2 kb PvuII fragment located on chromosome 8 [14]. This probe also identifies the *Emv-3* locus of DBA/2 as a 5.4 kb PvuII fragment located on chromosome 9 so DBA/2 DNA was also used as a control. The results of the hybridization are shown in Fig. 3. Lanes 1 and 2 show the 5.4 and 5.2 kb PvuII fragments from liver genomic DNA of DBA/2 and B10.F, respectively. Lanes 3, 4 and 5 contain PvuII digested DNA of liver, pooled peripheral and mesenteric lymph nodes, and spleen, respectively, of a B10.F mouse that was grey and had a low serum IgA. Several DNA fragments that hybridize with the MuLV probe are present in DNA from the lymph nodes and spleen.

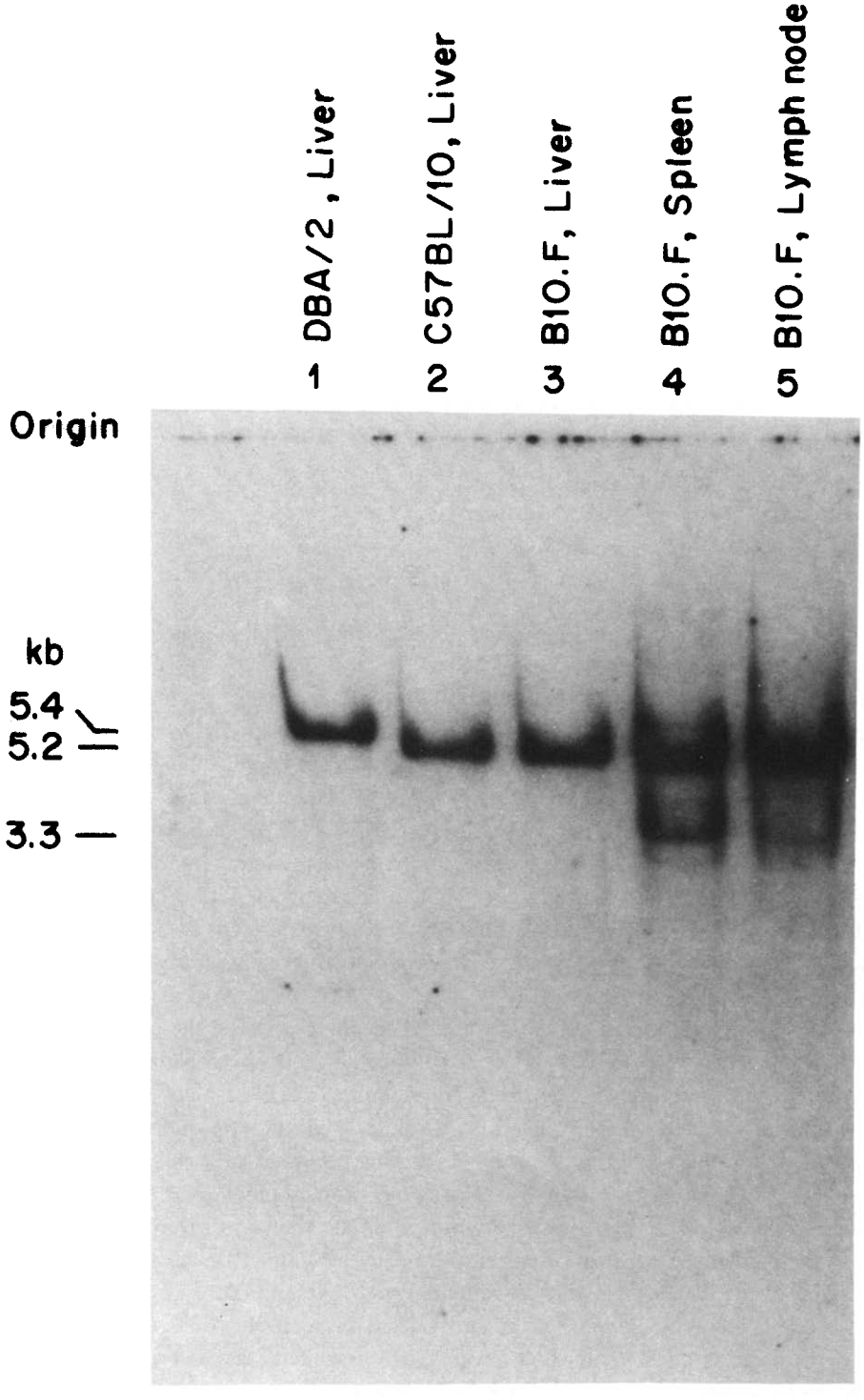

Fig. 3. Southern blot of PvuII digests of DNA from liver, spleen and mesenteric lymph node of a B10.F following hybridization with the 0.4 kb MuLV probe.

DISCUSSION

The data shown that among the offspring of the long-lived B10 and the short-lived B10.F mice the *H-2*$^{n/n}$ genotype is necessary but not sufficient to compromise lifespan. Only in combination with low levels of serum IgA is reduced survival observed. Thus, the previously reported maternal effect on the survival of progeny of reciprocal F_1 hybrid females [3] is due to the increased number of progeny with low IgA phenotypes among offspring of (B10.F × B10)F_1 females (Tables II–IV), particularly among the *H-2*$^{n/n}$ segregants: of the *H-2*$^{n/n}$ segregants from the F_2 and B10.F backcross matings with (B10.F × B10)F_1 females, 84% and 100%, respectively, have low serum IgA levels compared with 25% and 23%, respectively, of the *H-2*$^{n/n}$ segregants from similar matings with the (B10 × B10.F)F_1 females. Thus, the basis for *H-2* association with lifespan previously reported [3] is due to the combined effect of the appropriate *H-2* genotype (*H-2*$^{n/n}$) and the low serum IgA phenotype (Table V).

The one group of IgA low offspring in which no reduction in survival was seen (Table III) is from a mating in which no *H-2*$^{n/n}$ progeny are produced, the (B10 × B10.F)F_1 × B10 backcross. Because *H-2* segregates independently of IgA phenotype in this mating [4], 1/2 (5/11) of the progeny are homozygous for the *H-2*^{b} allele (all of which lived 638 days or longer) and the 50% survival of this group was 695 days. The remainder of the progeny (6/11) are heterozygous at *H-2*. Although the effect is not as severe, the presence of the *H-2*^{n} allele in the heterozygous state in association with low IgA levels also significantly reduces lifespan (Table IV). It would take a larger population for the lifespan reduction due to *H-2* heterozygosity to significantly reduce the survival of this backcross population. Because of the association of *H-2* and IgA phenotype found in progeny from the (B10.F × B10)F_1 female parent [4], the preponderance of the IgA low offspring from the B10 backcross with this female parent (7/8) are *H-2*$^{n/b}$ and half of the mice are dead by 600 days. Therefore, the greater proportion (1/2) of *H-2*^{b} homozygotes among the IgA low group from the (B10 × B10.F)F_1 female parent contributes to longer group survival. Conversely, the preponderance of *H-2* heterozygotes among the IgA low group from (B10.F × B10)F_1 female parent results in reduced group survival which is apparent even in small populations.

Several genetic models to explain a maternal effect have been suggested [4] and maternal physiology and the transmission of self-replicating particles were discussed. Short-term allotype suppression in rabbits and mice has been shown after inducing antipaternal-type immunoglobulin in the female parent [15]. However, long-term allotype suppression in mice was found to be brought about and maintained by cellular mechanisms [15] demonstrated by spleen cell transfers into neonates [7]. These experiments led to the suggestion that chronic allotype-specific suppression was induced by the transfer of suppressor cells to the immunologically immature neonate [15]. To assess an immunoregulatory role of maternal cellular components on IgA phenotype, we carried out several cell transfer experiments. Data show that neonatal offspring can be modulated to have low IgA serum levels by spleen cell transfer, foster nursing or bone marrow transfer (Tables V–VIII). The modulation was permanent throughout the lifespan of the

animal and could be passed by females to the next generation offspring. However, when blastocysts of potentially low IgA mice were gestated in the uterus of a high IgA female the mature offspring developed high serum IgA levels. The results of these experiments suggested that the basis of the IgA specific suppression is the presence of allotype specific suppressor cells in the B10.F mice and that suppressor cells are induced in F_1, and some F_2 and backcross progeny of suppressed females. The alternative hypothesis, i.e. transmission of a viral particle, is also consistent with the results of the cellular studies. The discovery of active virus shedding in the B10.F required additional experiments to distinguish between these alternatives. Modulation of IgA levels following transfer of a cell-free splenic filtrate established that the mechanism for the long-term suppression in neonatally injected or fostered-nursed offspring was not cell mediated.

The experiments described in this report are consistent with the suggestion that a vertically transmitted virus either inherited or acquired from B10.F and (B10.F × B10)F_1 mothers is responsible for the deficient serum IgA levels among their offspring. Yetter *et al.* [16] have reported that the B10.F mouse, in contrast to its B10 congenic partner, sheds an endogenous, ecotropic virus. Tests on our B10.F and B10 colonies confirmed this observation. In addition, we have found that although the genomic DNA of B10.F does not differ from B10 with respect to the chromosomal location of the integrated virus, multiple restriction fragments were found in the DNA of spleen and lymph nodes, suggesting that shed virus had reintegrated at additional sites in the DNA of some cells of these tissues (Fig. 3). These data indicate that MuLV reintegration occurs in a minority component of the heterogenous lymphoid population and that a differentiation-specific restriction system may govern the expression of the MuLV. The data also suggest an etiological role for the ecotropic virus in regulation of IgA levels and life shortening. However, other physiological differences may also be related to viral burden [17,18] and the target cell for viral reintegration may be random leading to a variety of phenotypes. Studies are under way to determine if *H-2*$^{n/n}$ is uniquely susceptible to the virus and if viral reintegration is associated with IgA phenotype and low survival of *H-2*$^{n/n}$ progeny. If so, the viruses shed by the B10.F mothers and passed on to their offspring cause a deficient IgA humoral response that compromises survival.

REFERENCES

1 D.M. Popp, Use of congenic mice to study the genetic basis of degenerative diseases in selected long-lived inbred mice and their F_1 hybrids. In D. Bergsma and D.E. Harrison (eds.), *Genetic Effects in Aging*, Alan R. Liss, New York, 1978, pp. 261–279.

2 D.M. Popp, Basal serum immunoglobulin levels. I. Evidence for genetic control in B10 and B10.F mice. *Immunogenetics*, 9 (1979) 125–135.

3 D.M. Popp, An analysis of genetic factors regulating life-span in congenic mice. *Mech. Ageing Dev.*, 18 (1982) 125–134.

4 D.M. Popp, Basal serum immunoglobulin levels. II. Integration of genetic loci and maternal effect on regulation of IgA. *Immunogenetics*, 9 (1979) 281–292.

5 P.A. Gorer and Z.B. Mikulska, The antibody response to tumor innoculation. Improved methods of antibody detection. *Cancer Res.*, 14 (1954) 651–655.

6 G. Mancini, A.O. Carbonara and J.F. Hermans. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, 2 (1965) 235–254.
7 E.B. Jacobsen and L.A. Herzenberg, Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F_1 mice. *J. Exp. Med.*, 135 (1972) 1151–1162.
8 J.W. Hartley and W.P. Rowe, Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. *Virology*, 65 (1975) 128.
9 W.P. Rowe, W.E. Pugh and J.W. Hartley, Plaque assay techniques for murine viruses. *Virology*, 42 (1970) 1136–1139.
10 R.W. Tennant, B. Schluter, W.K. Yang and A. Brown, Reciprocal inhibition of mouse leukemia virus infection by Fv-1 allele cell extracts. *Proc. Natl. Acad. Sci. USA*, 71 (1974) 4241–4245.
11 S.A. Aaronson and G.J. Todaro, Development of 3T3-like lines from Balb/c mouse embryo cultures. Transformation susceptibility to SV40. *J. Cell Physiol.*, 72 (1968) 141–148.
12 G.J. Todaro and H. Green, Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J. Cell. Biol.*, 17 (1963) 299.
13 S.K. Chattopadhyay, M.R. Lander, E. Randsan and D.R. Lowry, Structure of endogenous murine leukemia virus DNA in mouse genomes. *Proc. Natl. Acad. Sci. (USA)*, 77 (1980) 5774–5778.
14 N.A. Jenkins, N.G. Copeland, B.A. Taylor and B.K. Lee, Organization, distribution and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of Mus musculus. *J. Virol.*, 43 (1982) 26–36.
15 L.A. Herzenberg and L.A. Herzenberg, Short-term and chronic allotype suppression in mice. In M.D. Cooper and N.L. Warner (eds.), *Contemporary Topics in Immunobiology*, Vol. 3, Plenum Press, New York 1974, pp. 41–75.
16 R.A. Yetter, J.W. Hartley and H.C. Morse III, *H-2*-linked regulation of xenotropic murine leukemia virus expression. *Proc. Natl. Acad. Sci.*, 80 (1983) 505–509.
17 H.C. Morse III, R.A. Yetter, J.H. Stimpfling, O.M. Pitts, T.N. Fredrickson and J.W. Hartley, Greying with age in mice: Relation to Expression of murine leukemia viruses. *Cell*, 41 (1985) 439–448.
18 T. Imamura, D.H. Devens, H. Bruce, R. Weindruch and R.L. Walford, Drug metabolizing capabilities and drug-induced immunosuppression in two strains of H-2 cogenic mice. *Age*, 7 (1984) 42–46.
19 J. Klein, *Biology of the Mouse Histocompatibility-2 Complex*, Springer-Verlag, NY, 1975, p. 118.