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ROLE OF THE B COMPLEX IN THE GENETIC CONTROL OF ROUS SARCOMA VIRUS INDUCED TUMORS IN CHICKENS

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Role of the B complex in the

genetic control of Rous sarcoma virus

induced tumors in chickens

by

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INTRODUCTION

Lymphoid leukosis (LL) is a virus-induced lymphoblastic malignancy
originating in the bursa of Fabricius. This is a cancer-like disease and
affects almost any tissue of a chicken producing tumors. The disease is
initiated by an RNA group of a Myxo virus. Chickens are infected either
congenitally, through the egg (vertical transmission), or by direct
contact with infected chickens (horizontal transmission) (Siccardi and
Burmester, 1970).

LL has been responsible for one of the most costly disease problems
confronting the poultry industry in the past. Its seriousness today seems
to have considerably lessened. Purchase, et al. (1972), in a survey of
more than 145,000 birds over a period of approximately 18 months, reported
an average of 3.3% mortality due to LL. Lifetime losses from LL amounted
to 1.2 to 3.4% in experimental strains of Leghorns and meat-type breeders
(Gavora, et al., 1975). In large-field surveys conducted in Great Britain,
1.4% of all laying flocks (Randall, et al., 1977) and 1.5% in broiler
breeder flocks died from LL. In some flocks, much higher losses from LL
have been experienced (Jones, et al., 1978; Crittenden and Witter, 1978).

Recently, the effects of LL virus on production and mortality were
investigated in approximately 2000 Leghorn pullets in each of two consecu-
tive years (Gavora, et al., 1980). The pullets were from nine strains
developed in Ottawa, of which three were unselected control strains and
six were strains under selection for up to 27 generations for high egg
production and a complex of related commercially important traits. The
overall frequency of birds shedding LL virus or group specific antigens

into eggs (LL-S) was 3.9% in the selected strains and 18.5% in the control strains. The LL-S pullets produced 30 and 25 eggs less per hen-housed than the nonshedders in 1976 and 1977, respectively. Also, the LL-S birds reached sexual maturity later, produced smaller eggs at a lower rate, and their eggs had a lower specific gravity, indicating thinner shells. Mortality from all causes to 497 days of age was significantly higher in the LL-S birds (+14.8%) in 1976. The eggs from LL-S dams had 2.4% lower fertility and 12.4% lower hatchability. The rearing of large numbers of birds under crowded conditions leads to much bird-to-bird contact and a contaminated environment. Tumors became a major cause of death loss.

On the other hand, chickens have played a leading role in tumor virology because viruses causing cancerous growths were discovered and isolated from chickens many decades before they were recognized in other animal species. Chickens have a relatively short life cycle, produce a large number of offspring, and are easy to grow and maintain under laboratory conditions. Also, the embryonated chicken egg is very convenient and, therefore, commonly used as a host for the study of viruses.

Little did we know that the first recognized transmission of a solid tumor (sarcoma) in chickens using cell-free inocula reported by Peyton Rous in 1911, was to be of such importance in resolving the avian leukosis problem 50 years later. The significance of this work to medical science was belatedly recognized by the awarding of a Nobel Prize to Rous in 1966.

In addition, a most remarkable discovery in the field of avian immunogenetics has been the role of the major histocompatibility complex (MHC) in the genetic control of immune functions. The development of congenic inbred lines in the chicken is useful for studies of genetic

differences in the MHC. In the chicken, the acute skin graft rejection, the mixed lymphocyte reaction, cell-mediated immune response to viral oncogenesis, humoral immune response, and serologically-defined antigens are all under genetic control by the B complex.

The purpose of this study was to further investigate the role of B complex in the control and fate of Rous sarcoma viruses induced-tumors in the chicken. The significance of inbreeding and gene frequency on fixation of tv and rs genes has also been examined along with an attempt to answer the question as to whether there is a single locus linked to the B complex, which controls the genetic expression of all virus subgroups (single locus hypothesis) or whether the B complex carries separate loci each controlling resistance or susceptibility to tumors induced by different avian sarcoma virus subgroups. Finally, the question of genetic linkage of immune response locus (Ir-GAT), which controls antibody production to the amino acid polymer, GAT, and the RSV genes, which control tumor regression to RSV-subgroup A has been studied.

REVIEW OF LITERATURE

The B System and Histocompatibility

Thirteen autosomal blood group systems have been described thus far in chickens (Briles, 1962; Crittenden, et al., 1970). These are designated alphabetically: A, B, C, D, E, H, I, J, K, L, N, P and R. Among these, the B system (Briles, et al., 1950) occupies an exceptional position with regard to the complexity of its antigens (Briles, et al., 1950; Gilmour, 1959). The B system is highly polymorphic. Antigens are located on the surface of both erythrocytes and lymphocytes.

Shortly after its discovery attracted attention of the investigators that different genotypes of this system may influence viability, hatchability and a number of economically important traits (Gilmour, 1960), Schierman and Nordskog (1961) reported that the B locus controls transplantation immunity using the skin grafting technique. Thus, they identified B as the major histocompatibility locus in chickens. Jaffe and McDermid (1962) showed that B locus controlled splenomegaly. They identified it as the major GVH splenomegaly locus. Schierman and Nordskog (1963) showed that B also controlled lymphocyte foci development using the chorioallantoic membrane (CAM) test. They identified it as the major GVH-CAM pock locus. Miggiano, et al., (1974) used the B genotype to identify a mixed-lymphocyte-reaction (MLR) locus. They showed that the MLR is controlled by a separate locus closely linked to B locus. These results leave no doubt that the B blood group locus identifies with the major histocompatibility complex of the chicken.

The Structure of the MHC

The best known MHC is the H-2 system of the mouse. It consists of a complex of loci responsible for a number of traits (Klein, 1975). Serologically detectable H-2 antigens are determined by alleles of two loci, H-2K and H-2D. These loci are located in the end regions of the H-2 complex, and recombinants with a 0.5% frequency have been found between them (Shreffler, 1970). However, little is known of the number and arrangement of genes in the chicken MHC, although it is of great interest to compare the genetic organization of the MHC's of various species; for example there are differences in the number and arrangement of loci in the H-2 in the mouse and the HLA complex in man (Klein, 1975).

Schierman and McBride, (1969) noted the occurrence of unusually rapid skin rejection among a group of recipient-donor pairs matched for compatibility on the basis of B hemagglutination reactions. The exceptional recipient, a female, was originally typed as B^2B^3, but after investigation she was redesignated B^2B^4 to indicate the uniqueness of the new allele, classified earlier as B^3. When normal B^2B^3 heterozygotes received skin from the B^2B^4 female, the graft was tolerated in a manner characteristic of non-B histocompatibility antigens. Thus, all data presented in this first report were compatible with the aberrant type B^4, having resulted from a recombination between chromosomal segments coding for the B^2 and B^3 alleles.

The second report of a probable recombinant in the B system was by Halá, et al., (1975 and 1976). Three inbred lines CB (B^1B^1), CC(B^2B^2), and WB($B^{10}B^{10}$), homozygous for transplantation and erythrocyte antigens were used in a special cross (CC X CB) F_1 X WB to produce B^1B^{10} and

B^2B^{10} chicks to be grafted with skin from congenic grandparent lines CC

and CB. Typical segregation of B^1 and B^2 occurred in all but two of 1,206

chicks resulting from the mating. In addition to B^{10}, erythrocytes of each

of these exceptional chicks (males) were agglutinated by both reagents B^1

and B^2. Male no. 744, was identified as a cross-over both within the

region coding for seriological determinants, but outside the region coding

also for histocompatibility determinants. Halá, et al. (1976 and 1977)

designated the putative locus determining the SD and H antigens B-F and

the other determining other SD antigens as B-G (Fig. 1). In addition, the

immunochemical approach (Ziegler and Pink, 1976) and tolerance induction

(Hartmanová et al., 1977), has led to roughly the same conclusions. Studies

of Pink et al., (1977) indicate that the chicken B complex includes at

least three regions: B and B-L within the B-F region plus the original

B-G region (Fig. 1). Biochemically, the B-L region may be homologous to

Ia antigens. The evidence for a duplicate-like gene (as K-D in H-2) could

not be substantiated by Pink et al., (1978); they favor the single locus

hypothesis. However, cytogenetic and immunologic features of chicken

cells have been studied in an effort to relate the B-blood group locus to

a specific chromosome. According to a Bloom and Cole (1978), the B locus

is linked to the nucleolus organizer region (NOR) on one pair of acrocentric

chromosomes rank in the size and ranged from 15 to 18.

Immune response to various antigens in different animal species is

controlled by specific immune response (Ir) genes linked to the major

histocompatibility complex (Benacerraf and Katz, 1975). Similarly, the

MHC of the chicken exerts genetic control of immune response to various

antigens. Control of immune response by the B-complex has been reported

for <u>Salmonella</u> <u>Pullorum</u> bacterin (Pevzner, et al., 1973 and 1975), tuberculin (Karakoz, et al., 1974), 2, 4-dinitrophenol group conjugated to chicken gamma globulin (DNP-CGG) (Balcorová, et al., 1974), and the synthetic polypeptides (T,G)-A--L (Günther, et al., 1974 and Balcarová, et al., 1975), GAT (Benedict, et al., 1975), and GT (Koch and Simonsen, 1977). Recently, immune response to (L-glutamic acid^{60}, L-alanine^{30}, L-tyrosine^{10}) (GAT) has been shown to be controlled by a distinct Ir gene linked to genes coding for the B serologically-determined (SD) antigens (Pevzner, et al., 1978). Ir-GAT^1 and Ir-GAT^{19} alleles control low and high immune response to GAT, respectively. A recombinant between B^1B^1 and $B^{19}B^{19}$ proved to be a high responder to GAT but serologically testing B^1B^1. Thus, the crossover involved the SD region of B^1B^1 and the IR region of $B^{19}B^{19}$. This lead to the conclusion that immune response to GAT is controlled by the Ir-GAT gene which is linked to the SD locus of the B complex. The GAT locus evidently divides the B-F region into B sub-regions and IR (B-L in the Hala - pink model). The latter is an immune response region, containing the Ir-GAT locus (Pevzner, 1979) (Fig. 2).

The gene controlling the fate of tumors induced by Rous sarcoma virus (RSV) subgroup A was studied by Gebriel, et al., (1979). Birds carrying the dominant allele regress the tumor; homozygous recessive (progressors) develop large tumors and die. Their results showed that all the chickens developing tumors from inoculation of RSV-subgroup A lacked cellular resistance. The rate of tumor regression between the high and low GAT responders differed and was highly significant. Thus, it is evident that the locus controlling the fate of RSV-induced tumors is closely linked to the locus controlling immune response to GAT (Fig. 2).

The term, allo-aggression, first used by Jerne (1971), specifies certain cellular reactions resulting from encounters of allogeneic lymphocytes. This may occur both in vivo and in vitro, but is usually not provided by xenogeneic cells or, if so, only to a much smaller degree. Earlier, Simonsen (1975) proposed the existence of phylogenetic factors such that certain xenogeneic cells fail to induce GVHR. This led him to propose the existence of allo-aggression (AA) gene(s), which were thought to be component(s) of the MHC genome and control the allo-aggression reaction. The gene product(s) of the AA locus would appear to be expressed, not only in T cells, but also in the nucleated erthrocytes, which would then permit their detection by hemagglutination. If so, there would be no need to make a distinction between SD and LD determinants. The AA gene(s) product is present in both bursa cells and the thymus of bursec-tomized birds. Within the limits of our current knowledge, it seems likely that the AA locus forms a part of, or is identical with, the B region because the respective antibodies can be absorbed by erythrocytes. So far, recombinations between the B-L and B loci have not been observed.

A condensation of current information on the structure of the chicken MHC from Hala's laboratory is presented in Fig. (1). Based on Pevzner's and Gebriel's studies at Iowa State University, a new linear order of genes in the B-complex can be proposed as in Fig. (2). The SD region may consist of two loci, as in the human HLA system, but in chickens, this has not yet been demonstrated. The IR region includes the Ir-GAT locus and possibly also the (R-Rs, r-Rs) tumor-regressing locus.

B complex		

Region (determined by crossing-over)	B-F		B-G

Loci (determined by biochemical analysis)	B	B-L	B-G
WBC agglutination	+	+	-
RBC agglutination	+	-	+
Antigen found on	RBC lymphocyte	lymphocyte	RBC
Analogous regions in the mouse H-2	K (or D)	IA	0
Antigenic effects	SD determinants H determinants MLR determinants		SD determinants
Line Origin	CB		CC

Figure 1. Structure of the chicken major histocompatibility complex (from Hála et al., 1977)

B complex

Region (determined by crossovers)	SD	IR	B-G

Genes	B	Ir- GAT	Rs rs

	──x────────x────────x────────────		
WBC antigens	+	+	-
RBC antigens	+	-	+

Figure 2. B-complex: a linear order of genes (from Pevzner, 1979; Gebriel et al., 1979)

Genetics and Control of Lymphoid Leukosis Viruses

The avian leukosis-sarcoma viruses have been divided into five sub-groups A, B, C, D and E (Vogt, et al., 1967; Duff and Vogt, 1969; and Hanafusa, et al., 1972). The main principles involved in applying genetic resistance to the control of lymphoid leukosis disease and the actual and potential importance of this approach to disease control are considered in terms of the following: (1) mechanisms of resistance, including virus receptors, (2) recognition of resistance, (3) role of cell mediated immunity, (4) inheritance of resistance, including simple and polygenic control, and (5) selection for resistance (Gyles and Brown, 1971; and Payne, 1973).

Mechanisms of resistance

The chicken has developed a variety of mechanisms for resistance which are under genetic control. Resistance, that is, the ability to withstand the pathogenic effects of the parasite, is usually highly specific to the parasite in question. The ability of avian leukoviruses, such as Rous sarcoma virus (RSV) and lymphoid leukosis virus (LLV), to infect chicken cells is dependent on the presence on the cell membrane of specific virus receptors necessary for uncoating of adsorbed virus (Piraino, 1967). Single recessive autosomal genes code for absence of receptors for leukoviruses of each of subgroups A, B, C and possibly D and E (Payne, 1973). If the block to the uncoating process is by-passed, resistant cells are found to support virus replication (Crittenden, 1968). Vogt and Ishizaki, (1965) suggested a phenotypic nomenclature based on the terminology for host bacterium resistance use by bacteriophage workers. The phenotypes of the cells are designated C/O, C/A, and C/A,B. The C stands for "chicken" and the bar for "resistant to" and the A and B represent the excluded virus subgroups. The phenotypes are controlled by two independent autosomal loci, called the tumor virus a (tva) and the tumor virus b (tvb) loci. The genes controlling susceptibility (a^s, b^s) appear to be dominant over the alleles controlling resistance (a^r, b^r) (Crittenden, et al., 1967).

Two levels of genetic resistance to lymphoid leukosis viruses are recognized (Crittenden, 1975). The first is cellular resistance, i.e., a mechanism that prevents the virus from penetrating living host cell. It has been clearly shown that cellular resistance to subgroups A, B and C is controlled, in each case, by a single autosomal recessive gene. Thus,

the tva locus controls resistance to subgroup A viruses with suscepti-
bility being completely dominant to resistance. The second line of
defense involves a mechanism of genetic resistance to tumor growth which
comes into play after the virus has penetrated the cell. In addition,
studies on eight inbred lines homozygous for B^1, B^{13}, or $B^{15.1}$ blood types
confirm that the genetic mechanism for cellular resistance is not
associated with the MHC (Gebriel and Nordskog, 1980).

Recognition of resistance

Certain genes with an easily observable phenotypic expression called
marker genes, may be associated with some other trait. For example,
differences in alleles at the B blood group locus have been associated
with differences in susceptibility to Marek's disease (MD) (Hansen, et al.,
1967; Brewer, et al., 1969). The reason for the association has not been
fully elucidated although Nordskog (1972) suggested the association of
viability with B blood group differences as being due to linkage.
Crittenden, et al. (1970) found an association between the R blood group
antigen and the tvb locus controlling susceptibility to subgroup B
leukoviruses. Pani, (1974) found a 4-fold resistance to RSV to be associ-
ated with the presence of the homozygous recessive black plumage gene i^+.

Recent studies of Collins, et al., (1977); and Schierman, et al.,
(1977) independently showed that regression of Rous sarcoma virus (RSV)-
induced tumors was a dominantly inherited trait controlled by a gene
within, or closely linked to, the B MHC. More recently, Marks et al.
(1979) studied the incidence of tumor regression in two highly inbred
lines of East Lansing White Leghorns, 6_3 and 7_2 plus their F_1 and

reciprocal backcross progeny. These lines evidently do not differ in their serological or histocompatibility alloantigens associated with the B blood group locus. In addition, Collins, et al., (1980) demonstrated marked genetic differences in tumor regression between inbred lines which share similar, if not identical, B locus erythrocyte alloantigens so that other unknown genes are probably also involved. However, Gebriel, et al., (1979) showed that the (Rs, rs) locus is closely linked to the Ir-GAT locus and, therefore, maps within or close to the Ir-region of the B MHC. Such markers are of interest as possible criteria for detecting and selecting disease-resistant fowl.

Role of cell-mediated immunity

Alterations in cell-mediated immunity (CMI) have been associated with oncogenesis. Cell-mediated immune cytotoxicity was lower in avian leukosis virus infected chickens with overt disease, but was normal in sub-clinically infected chickens (Granlund and Loan, 1974). Thymectomy did not alter the course of lymphoid leukosis in chickens (Peterson, et al., 1964). In addition, antithymus serum prevented development of Marek's disease lesions following virus inoculation (King, et al., 1972). The growth of Rous sarcomas was enhanced by immunosuppression (Halleux, 1971; Law, et al., 1968; and Sjogren and Borum, 1971).

Findings by Cotter, et al., (1975) were compatible with the suggestion of Rubin (1962) that Rous tumor regression and the restriction of meta-stasis of such tumors, are controlled by T-cell-mediated immune response. Radzichovskaja (1967a and b) has shown that Rous virus tumor growth was stimulated in thymectomized chickens but unaffected in bursectomized

chickens. Recently, Vlaovic, et al., (1977) concluded that the immuno-suppression of chickens infected in ovo with avian leukosis virus (RSV-1) by the injection of antilymphocyte serum did not significantly ($P < 0.05$) inhibit tumor regression but rather enhanced tumor metastasis.

Inheritance of resistance

Control of inheritance of resistance can be classified as single gene control or as polygenic control. In single gene control the susceptibility or resistance traits are clearly defined with no inter-mediate forms, and conform to simple Mendelian segregation ratios. The best example of single gene inheritance of disease resistance in fowl is the control of susceptibility of cells to infection by RSV or LLV of subgroups A, B and C. Resistance is controlled by single autosomal recessive genes (Crittenden and Okazaki, 1965; and Payne and Biggs, 1966). These studies were made in highly inbred lines of chickens, the use of which greatly simplifies the genetic analysis of qualitative traits by avoiding the complication of heterozygosis at individual loci. Neverthe-less, inbred stock are not essential for detection of single gene effects, as exemplified by the analysis by Pani and Biggs (1973) of response to RSV of F_1 progeny from two noninbred commercial lines. As with inbred birds, they found a major genetic influence of genes at a single locus, accounting for 53% of the total variance compared with 0.7% due to genes at other loci.

When a trait is controlled by more than a few pairs of genes, it becomes difficult to identify distinct phenotypes. This is due to the cumulative effects of many genes. The estimate of the proportion of

variation in a population which is of additive genetic origin and respon-
sible for resemblance between relatives, and on which selection is based,
is termed heritability (h^2); it is defined as the ratio of additive
genetic variance to total phenotypic variance, VA/VP (Falconer, 1960).
Some examples of h^2 calculated for disease resistance are: leukosis
(Lush, et al., 1948) 0.058; lymphomatosis (Robertson and Lerner, 1949)
0.048; Marek's disease (Von Krosigk, et al., 1972) 0.10 - 0.20; and Rous
sarcoma (Pani and Biggs, 1973) 1.59; (Bower, 1962) 0.28.

In White Leghorn strains of fowl, it has been shown that the two
loci, tva and tvc for cellular resistance are linked (Payne and Pani,
1971; Pani, 1974). This has recently been confirmed in the Rhode Island
Red breed (Dren and Pani, 1977). The linkage value of 0.22 in the male
sex agreed well with the reported in White Leghorn male sex, indicating
that the two loci are located in the same sites in homologous chromosomes
in the two breeds. However, in the Rhode Island Red, no sex differences in
crossing over between the two linked loci were found.

On the other hand, Crittenden, et al., (1972) concluded that the
genetic resistance of chickens to lymphoid leukosis is a highly complex
trait, but that at least one simply inherited mechanism of resistance
exists which acts at the level of virus infection. Other complex factors
govern the bird's ability to cope with this infection. In addition,
Schierman, et al., (1977) found that regression of RSV tumors, induced
by the Schmidt-Ruppin strain of subgroup B, is determined by a dominant
gene designated R-Rs-1. The recessive allele designated r-Rs-1, permits
uncontrolled (progressive) tumor growth in homozygous birds.

Selection

The main systems of artificial selection include mass selection, family selection, combined selection utilizing information from both individual phenotypes and families and various techniques to select for multiple characters simultaneously e.g., selection index. Several investigators have conducted selection studies for resistance and suscep-tibility of chickens to avian leukosis. McClary, et al., (1951); Waters (1951); and Bears, et al., (1963) all reported varying degrees of success in selecting divergent lines, from the same original stock for resistance and susceptibility to avian leukosis complex. The duration of these selection experiments ranged from four to twenty-eight generations. The basis of selection most often used was full-sib family selection, although progeny testing was employed in some studies. The criterion of selection was the percentage of mortality from all subgroups of leuko-viruses during the rearing and laying periods. Natural exposure was usually the sole means of challenge. In a few instances, tumor material was placed in the drinking water.

The most striking example of the results of progeny testing for selecting susceptible and resistant stock is that of Cole (1968 and 1972). By determining the progeny response to inoculated MD tumor cells, he selected, in four generations, a resistant line with 6.5% susceptibility to MD and a susceptible line with 94.4% susceptibility from common stock with an average susceptibility of 51.1%. Progeny testing, used under commercial conditions to select for MD resistance, has been reported by Friars, et al., (1972) and by Von Krosigk, et al., (1972); both groups reported significant progress after one generation of selection.

Gyles and Brown, (1971) studied the effect of family selection on tumor regression induced by RSV. A closed population of chickens, initiated primarily by F_1 and F_2 crosses between Arkansas Experiment Station strains of White Leghorn and Giant Jungle Fowl, was selected for regression of Rous sarcomas for six generations. Birds were challenged in the wing web with RSV at approximately six weeks of age, scored and observed weekly for size and regression of tumors. The percentage of birds inoculated that exhibited regression of tumors increased from 14.3 to 59.2 and the percentage of tumors initiated that regressed increased from 18.2 to 63.7 over six generations of selection.

MATERIALS AND METHODS

Genetic Stock

Inbred Lines

Eight highly inbred lines, with inbreeding coefficients ranging from 81% to 97% and segregating for eight different B locus alleles were used in this study. They were developed and maintained at the Poultry Research Center, Iowa State University, Ames, Iowa. Some of them have been closed since 1940 (Table 1).

Line 8: A barred plumaged Leghorn about 91% inbred. Its origin is the same as lines 9 and 19 (Waters and Lambert, 1936). It segregates for the sex-linked barring gene, and the (I, i) dominant gene for white plumage color. In the 1950s it was maintained by mass mating for eight generations and since then has been pedigree mated (Marangu, 1970). Two B alleles, B^1 and $B^{15.1}$, segregate. B^1 was transferred from line S1 and the $B^{15.1}$ is identical to the $B^{15.1}$ of line 9 (Somes, 1978).

Line 9: A Leghorn line inbred to about 89% with forced segregation for plumage color. In 1956 line 9 was crossed to the Spanish line having black plumage and successively backcrossed six times to line 9 maintaining plumage color segregation (Smith and Nordskog, 1968). It is homozygous for the $B^{15.1}$ blood group allele.

Line 19: A white Leghorn about 90% inbred originating from crosses tracing back to old Iowa State University inbreds maintained in the 1930s. It seems to be fixed for most blood group loci but segregates for three B alleles, B^1, B^{13} and $B^{15.1}$. B^1 was transferred from line S1 in 1973.

Line GH: A white Leghorn about 90% inbred, originating from a commercial outbred strain (Ghostley). They are descendents of a female which had been

accidentally mated to an HN male in 1958 producing F_1 progeny. An F_1 male was later mated to a line GH female and also to two full-sibs so that back-crosses and F_2 progeny were obtained. In subsequent generations, matings were designed to maintain segregation at the B and C blood group loci (Schierman, 1962). This line has been used extensively in skin trans-plantation studies; three B alleles, B^{13}, $B^{15.1}$ and B^1, have been kept segregating. The latter is the same B^1 allele found in the S1 line.

Line GHs: A subline of GH maintained since 1965 at the New York Medical College and later at the University of Georgia by Dr. L. W. Schierman. In 1976 a sample was returned to Ames. It segregates for two B alleles, B^6 and B^{13} (Trowbridge et al., 1978).

Line HN: A white Leghorn line, about 97% inbred; originally obtained as a pure Kimber line from Heisdorf-Nelson in the early 1950s. It has been used for skin grafting experiments and segregates at the B blood group locus. Three congenic lines with blood group alleles, B^1, B^{12} and B^{15}, were utilized for this study. B^1 allele was transferred from line S1.

Line SP: Spanish, a breed originating in Spain and brought to Iowa State in 1954, about 92% inbred. It segregates for two B alleles, B^1 and $B^{21.1}$. B^1 allele was transferred from line S1.

Line M: Fayoumi, a line derived from a breed of the same name imported from Egypt in 1949 primarily to test its resistance to lymphoid leukosis disease. About 81% inbred, segregating for alleles B^Y, B^Z and B^1.

Outbred line

Outbred line S1 produced from a cross of two Hy-line inbred lines in 1964 (Nordskog, et al., 1973). It segregates for the blood group alleles, B^1, B^2 and B^{19}. A subpopulation of the S1 line was subjected to 2-way

selection for high and low antibody titer after inoculation with <u>Salmonella</u>

<u>pullorum</u> bacteria (Pevzner et al., 1978). Within each genotype, B^1B^1

B^2B^2 and $B^{19}B^{19}$, divergent selection for <u>S</u>. <u>pullorum</u> titer was continued

over four successive generations. In the second generation, breeders

were tested for immune response to GAT. The fourth generation progeny of

the B^1B^1 genotype came from four mating types: Hi-Hi, Hi-Lo, Lo-Hi and

Lo-Lo with respect to GAT response and <u>S</u>. <u>pullorum</u> titer. In addition,

B^2B^2 intermediate, $B^{19}B^{19}$-GAT-Hi and $B^{19}B^{19}$-GAT-Lo, were included in this

experiment. Divergent selection for <u>S</u>. <u>pullorum</u> titer within these

genotypes did not prove successful. Thus, essentially all B^2B^2 and $B^{19}B^{19}$

birds used in this study were high (or "normal") responders to <u>S</u>. <u>pullorum</u>.

Heavy breed line

Line W is a heavy-breed population synthesized from a cross between

the Rhode Island Red and the Barred Plymouth from a cross in 1954. It is

large in body size and has gold-barred plumage. This breed is highly

resistant to lymphoid leukosis. In the summer of 1977, based on a pre-

liminary CAM test the line was divided into two sublines, WR and SW in

an attempt to establish resistant and susceptible to RSV-subgroup A,

respectively. At present this dichotomy, however, has been only partially

successful. The WR and WS lines, estimated to have inbreeding coefficients

of about 40%, have never been blood typed. These are brown-egg lines

and may or may not carry distinctly different B locus haplotypes.

<u>Virus strains</u>. Purified stocks of RSV were used. Of practical

significance is that the host range of RSV coincides with that of lymphoid

leukosis viruses of the same subgroups. Moreover, RSV is easily assayed

on the chorioallantoic membrane (CAM) (Vogt, 1965). Bryan high titer RSV

(BH-RSV) with Rous associated virus (RAV) were kindly supplied by Dr. L. B. Crittenden, RPRL, East Lansing, Michigan. The RSV-subgroups and dilutions of each used were

Subgroup	Pseudotype	Abbr.	ffu/ml[1]	Dilution Wingweb	CAM
A	BH-RSV(RAV-1)	RSV-1	1×10^5	1-200	1-25
B	BH-RSV(RAV-2)	RSV-2	5×10^5	1-400	1-50
C	BH-RSV(RAV-7)	RSV-7	1×10^5	1-100	1-15

Wing web inoculation and tumor measurement. A total of 1,768 birds were inoculated in the subcutaneous tissue of the left wing web at 5 weeks of age with a dosage of 0.05 ml per chick using a 26-gauge 3/4 inch needle. All birds were examined once per week from the 7th to the 70th day post-inoculation. Tumor response, induced by RSV, was scored on a scale ranging from zero (no tumor) to 6 (a massive tumor) as per Collins et al. (1977). Tumors developing within the intervals from 7 to 28, 29 to 49, and 50 to 70 days were designated arbitrarily as early, intermediate and late period, respectively (Gebriel et al., 1979).

Chorioallantoic membrane inoculation and observations. A total of 966 embryos were inoculated in the dropped chorioallantoic membrane (CAM) on the 12th day of incubation (Groupe et al., 1957). On the 19th day they were removed from the incubator and placed in a cold room at 2 to $4^\circ C$ for observation in the following day. The inoculum consisted of 0.1 ml of a virus dilution tested to yield at least 100 pocks on the CAM's of susceptible embryos. Pocks on the membranes were counted individually and

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[1]Focus forming units per ml.

classified as negative (no pocks) or as positive (> 0-25, 26-50, 51-75, and over 75 pocks).

Terminology. The gene designations for cellular resistance-susceptibility to the various Rous sarcoma subgroups and for tumor regression-progression are:

Virus subgroups	Genes for cellular res/suc		Genes for tumor regr/progr	
	Dominant	Recessive	Dominant	Recessive
RSV-A	Tv-A^S	tv-A^r	R-Rs-BT^1	r-Rs-BT
RSV-B	Tv-B^S	tv-B^r	R-Rs-BT	r-Rs-BT
RSV-C	Tv-C^S	tv-C^r	R-Rs-BT	r-Rs-BT

The designation for cellular res/susc follows that in the Biological Handbook III (Altman and Katz, 1979). The allelic designations, R-Rs-1 and r-RS-1, as proposed by Schierman et al. (1977) designate a dominant regressor and a recessive progressor allele for locus 1. Their study was confined to the Schmidt-Rupin virus subgroup B. For simplicity we shall use "tv" and "rs" to designate, in general, any cellular res/susc locus and the tumor regr/progr locus, respectively. The existence of one B complex-linked locus which can be occupied by a series of alleles. In particular, I shall use R-Rs-12, r-Rs-1, and r-Rs-15 to designate one dominant and two recessive alleles linked, respectively, to the B^{12}, B^1, and B^{15} blood group alleles.

Statistical analysis. The data were analyzed by the Chi-squared test with a correction for continuity when the criterion has a single degree of

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[1]Blood type.

freedom (Steel and Torrie, 1960). Gene frequencies (Table 2) were estimated for both the tv and rs genes by application of the Hardy Weinberg equilibrium law (Falconer, 1960). In particular, for a total of N birds inoculated with, say, virus subgroup A we define:

$$np = \text{number of progressors}$$

$$nr = \text{number of regressors}$$

$$nt = np + nr = \text{number developing tumors}$$

$$nf = \text{number giving a negative response}$$

$$N = nt + nf = np + nr + nf$$

The fraction, nf/N, represents the proportion of recessive birds of, say, the genotype, $tv\text{-}A^r\ tv\text{-}A^r$, which possess cellular resistance to RSV subgroup A infection. The estimated frequency of the $tv\text{-}A^r$ gene is $q = \sqrt{nf/N}$ and that for the $Tv\text{-}A^s$ allele is $p = 1 - q$.

The fraction, np/nt, represents the observed proportion of birds of, say, the recessive genotype, $(r\text{-}Rs)(r\text{-}Rs)$, which develop progressive tumors. The estimated frequency of the $r\text{-}Rs$ gene is $q' = \sqrt{np/nt}$ and of the allele, $R\text{-}Rs$, is $p' = 1 - q'$. These statements are summarized in Table 2 which also shows the expected frequency of the 4 types of gametes in the population. Thus, the frequency of the double recessive gamete, $tv\text{-}A^r\ r\text{-}Rs$, is pq'. Note that:

$$p + q = 1 = p' + q' \text{ and, therefore,}$$

$$pp' + q'p = (p' + q')\, p = p \text{ and}$$

$$p'q + q'q = (p' + q')\, q = q$$

Likewise:

$$p'p + p'q = p'\, (p + q) = p'$$

$$q'p + q'q = q'\, (p + q) = q'$$

Table 1. Homozygous B blood types derived from 8 inbred lines, one out-
bred line and heavy breed used in this study

Line	Breed	Plumage	Year of Origin	% inbreeding 1978	Segregating Ea-B blood type
8	Leghorn	Barred	1940	91	B^1 and $B^{15.1}$
9	Leghorn	Colored	1940	89	$B^{15.1}$
19	Leghorn	White	1940	90	B^1, B^{13} and $B^{15.1}$
GH	Leghorn	White	1954	90	B^1, B^{13} and $B^{15.1}$
GHs	Leghorn	White	1954	95	B^6 and B^{13}
HN	Leghorn	White	1954	97	B^1, B^{12} and B^{15}
SP	Spanish	Black	1954	92	B^1 and $B^{21.1}$
M	Fayoumi	Silver-penciled	1954	81	B^1, B^Y and B^Z
S1	Leghorn	White	1962	Outbred	B^1B^1-GAT-Lo
					B^1B^1-GAT-Hi
					B^2B^2 intermediate
					$B^{19}B^{19}$ GAT-Lo
					$B^{19}B^{19}$ GAT-Hi
WR	Heavy-breed synthetic	Red-barred	1960	40	WR
WS	Heavy-breed synthetic	Red-Breed	1960	40	WS

Table 2. Expected frequency of cellular resistant (Tv) and tumor
regression (Rs) genes

Tumor	Cellular susc/resist		Sum
	susc (Tv)	resist (tv)	
Progr (rs)	pp'	pq'	p
Regr (Rs)	qp'	qq'	q
	$\overline{p'}$	$\overline{q'}$	$\overline{1}$

RESULTS AND DISCUSSION

Multiple Alleles at a Single Locus

Linked to the B Complex

Incidence of tumor response induced from wing web inoculation of RSV-A is given in Table 3. Presumably, part of the variation is due to sampling error, although differences in tumor regression among B complex genotypes are highly significant, statistically. For cellular resistance, the percentages ranged from 8.0 to 21.4 and from 10.0 to 24.0 in the first and second hatches, respectively. For tumor regression, the percentages ranged from 11.1 to 93.3 in the first and from 10.5 to 100.0 in the second hatches. The incidence of tumor response among B complex genotypes in the first hatch agrees closely with the results of the second, i.e., 14.8 vs. 19.4%. Also the mean values of the homozygotes vs. heterozygotes genotypes were almost the same, i.e., 14.7 vs. 19.0%. For tumor regression, (Figure 1) the percentages were 51.0 and 42.5 for hatch I vs. hatch II, respectively, and 43.7 and 50.0 for homozygous vs. heterozygous genotypes, respectively. Differences among genotypes in cellular resistance were within sampling errors. In tumor regression, $B^{12}B^{12}$ was the highest (96.8%) and B^1B^1, $B^{15}B^{15}$, and B^1B^{15} had the lowest percentages (11.1, 15.8, and 14.6, respectively). The B^1B^{12} and $B^{12}B^{15}$ genotype were intermediate (87.5 and 60.0%, respectively)

Chi-squared differences among the six B complex genotypes over both hatches are presented in Table 4. Differences in cellular resistance were not significant, but for tumor regression, B complex differences within both homozygous and heterozygous genotypes were highly significant. These

results verify findings reported by others (Schierman et al., 1977;

Collins et al., 1977; Gebriel et al., 1979) that tumor regression, but

not cellular resistance, is controlled by genes linked to the B complex.

Table 5 and Figure 1 summarize the results showing mean differences

among hatches, "zygotes" and genotypes in "progressor" tumor development.

For $B^{12}B^{12}$, 30 of 31 tumors proved to be regressors; only one was

classified as a progressor tumor. The allele linked to the B^{12} haplotype,

designated R-Rs-12, is, therefore, a regressor gene.

For $B^{1}B^{1}$, of 18 tumors, 16 were progressors and only 2 were regressors.

Thirteen of the 16 tumors (81%) were formed in the early period. Thus,

most B^{1} haplotypes carry a progressor gene r-Rs-1 which proved to be

almost completely recessive to R-Rs-12 of B^{12}. Thus, as expected for the

$B^{1}B^{12}$ heterozygote genotype, 28 of 32 tumors were of the regressor type

and only 4 were progressors.

For birds of the $B^{15}B^{15}$ genotype, 32 of 38 tumors were progressors.

The B^{15} haplotype, therefore, carries a gene similar, if not identical to

that linked to the B^{1} haplotype. However, 25 of the 32 progressor tumors

(78%) were late developers in contrast to the $B^{1}B^{1}$ linked gene where 81%

developed early.

Examination of the results of the $B^{12}B^{15}$ heterozygous genotype reveals

that 9 of 10 progressive tumors were late formers contrasting with the

$B^{1}B^{12}$ heterozygotes where the 4 progressor tumors developed early. Thus,

it seems that the r-Rs-15 allele carried on B^{15} is similar to that carried

on B^{1} in that they both are recessive to R-Rs-12 linked to B^{12} except that

the latter is incompletely dominant to r-Rs-15 but almost completely

dominant to r-Rs-1. Also, as already noted, the B^{15}-linked allele produces

late developing progressive tumors and the B^1-linked allele produces early developing progressive tumors.

Finally, a comparison of tumor expression of the B^1B^{15} heterozygote with the 2 corresponding homozygotes (B^1B^1 and $B^{15}B^{15}$) reveals that tumor expression of the B^1B^{15} heterozygote is essentially the same as the $B^{15}B^{15}$ homozygote, i.e., for B^1B^{15}, 27 of 35 progressive tumors (77%) were late developers and for $B^{15}B^{15}$, 25 of 32 (78%) were late developers. Thus, late development, as expressed by the $B^{15}B^{15}$ genotype, is seemingly dominant to early development as expressed by the B^1B^1 genotype.

Table 6 presents the observed vs. the expected number of regressor/progressor-type tumors formed in birds of the 2 heterozygous genotypes $B^{12}B^{15}$ and $B^{12}B^1$. The expected numbers are based on calculations using allelic frequency estimations taken from the homozygotes B^1B^1, $B^{12}B^{12}$, and $B^{15}B^{15}$. The results demonstrate that the alleles linked to B^{12}, i.e., (R-Rs-12), and to B^1, i.e., (r-Rs-1), behave essentially as a fully dominant and recessive gene pair. The nonsignificant χ^2 test demonstrates that the observed ratio of regressor/progressor tumors agrees closely with expectation. On the other hand, the allelic pair linked to B^{12} and B^{15} demonstrates that the B^{15}-linked gene is not fully recessive to the B^{12} linked allele. Thus, for the heterozygote $B^{12}B^{15}$ in Table 3, we note that the χ^2 for the combined hatches is highly significant, statistically. Therefore, the two alleles, respectively, linked to the B^1 and B^{15} haplotypes, are more or less recessive to the B^{12}-linked allele but differ one from the other. This leads us to deduce that the B complex-linked genes controlling tumor expression belong to a multiple allelic series.

The consensus is that alleles existing in only 2 alternative states

are more the exception than the rule (Hartl, 1980). For tumor development

genes determining sarcoma-leukosis resistance/susceptibility, however, only

2 alleles are, so far, reported for susceptibility/resistance to virus

subgroups A (Tv-A), C (Tv-C), and E (Tv-E). On the other hand, 3

susceptible alleles and one resistant allele have been reported for

virus subgroup B (Tv-B). It might be expected, therefore, that additional

alleles should be discovered for RSV-induced tumor regression for each

of the virus subgroups.

The discovery of a distinct genetic mechanism linked to the B complex

controlling tumor regression is rather recent (see literature cited in

Introduction), although Gyles et al. (1968) was the first to suggest tumor

regression was controlled by a single gene. Schierman et al. (1977)

proposed a nomenclature already used in mouse studies. He proposed R-Rs-1

and r-Rs-1 as designations for a dominant regressor allele found in his

line G-B2 and r-Rs-1 to designate a recessive progressor allele found in

line G-B1. It has been demonstrated by Trowbridge et al. (1978) and

in our laboratory that line G-B1 carries the B^{13} allele using a reference

reagent standard proposed by an ad hoc committee and Schierman's line G-B2

carries the B^6 allele. It would seem useful, therefore, to relate these

tumor regression genes to the B complex blood type to which they are linked.

Thus, Schierman's R-Rs-1 and r-Rs-1 allelic pair might be more meaningfully

designated R-Rs-6 and R-Rs-13. As already indicated, for the alleles

linked to the B^1, B^{12}, and B^{15} complexes in this study, we propose the

symbols r-Rs-1, R-Rs-12, and r-Rs-15.

The simplest hypothesis of tumor regression control is that there

exists an allelic series of multiple alleles at a single B complex-linked

locus. These alleles are assumed to respond more or less uniformly to tumors initially arising from Tv, tv susceptibility/resistance genes for all subgroup viruses (see Altman and Katz, 1979).

An alternative, but more complex hypothesis, is that there exists a series of tumor regression loci, all linked to the B complex, each of which controls tumor expression uniquely for each of the tumor virus subgroups, A, B, C, etc.

It is recognized that there may exist recombinant forms of B complexes. Thus, Gebriel et al. (1979) showed that genes for tumor expression are closely linked to the locus controlling immune response to GAT. In particular, they found that B^1B^1 GAT-Low birds were almost all progressors but progeny of B^1B^1 GAT-High matings contained both regressors and progressors. In this case, allelic designations would be R-Rs-1 and r-Rs-1 implying that B^1 haplogypes exist in recombinant form. More recently, Collins et al. (1980) demonstrated the existence of both regressor and progressor type alleles linked to the B^2B^2 complex. Nevertheless, the inclusion of the B blood type in a uniform nomenclature would be useful because of the tight linkages of tumor regression genes to the B complex.

Table 3. Tumor response from wing web inoculation of RSV subgroup A within B complex genotypes of the HN inbred line

B Complex Genotype	Cellular Resistance			Tumor Regression		
	No. Inoculated	No. Negative	%	No. Tumors	No. Regressors	%
First Hatch						
B^1B^1	10	1	10.0	9	1	11.1
$B^{12}B^{12}$	16	1	6.3	15	14	93.3
$B^{15}B^{15}$	22	4	18.2	18	3	16.7
B^1B^{12}	25	2	8.0	23	21	91.3
B^1B^{15}	28	6	21.4	22	4	18.2
$B^{12}B^{15}$	14	3	21.4	11	7	63.6
Second Hatch						
B^1B^1	10	1	10.0	9	1	11.1
$B^{12}B^{12}$	18	2	11.1	16	16	100.0
$B^{15}B^{15}$	26	6	23.1	20	3	15.0
B^1B^{12}	11	2	18.2	9	7	77.8
B^1B^{15}	25	6	24.0	19	2	10.5
$B^{12}B^{15}$	18	4	22.2	14	8	57.1

Table 4. Chi square tests for significance of tumor response from
wing web inoculation of RSV-A in HN inbred line over two
hatches

Source of Variance	d.f.	χ^2 Cellular Resistance	χ^2 Tumor Regression
Hatches	1	0.57	1.23
Homozygous vs. heterozygous	1	0.47	1.73
Hatch x "zygotes"	1	0.01	1.13
Blood types (BT) within homozygotes	2	2.66	55.61**
Blood types within heterozygotes	2	2.02	39.52**
Hatch x BT within homozygous	2	0.13	3.16
Hatch x BT within heterozygous	2	0.09	3.46

**$P \leq 0.01$.

Table 5. Mean tumor response summarized by hatch, "zygotes" and serotypes

	No. Inoculated	No. Tumor	Tumor Regression		Tumor Progression			
			No.	%	No.	Early	Inter.	Late
Summary by experiments:								
First hatch	115	98	50	51.0	48	12	6	30
Second hatch	108	87	37	42.5	50	11	8	31
Summary by genotypes:								
Homozygous genotype	102	87	38	43.7	49	15	9	25
Heterozygous genotype	121	98	49	50.0	49	8	5	36
Summary by serotypes:								
$B^1 B^1$	20	18	2	11.1	16	13	3	0
$B^{12} B^{12}$	34	31	30	96.8	1	1	0	0
$B^{15} B^{15}$	48	38	6	15.8	32	1	6	25
$B^1 B^{12}$	36	32	28	87.5	4	4	0	0
$B^1 B^{15}$	53	41	6	14.6	35	4	4	27
$B^{12} B^{15}$	32	25	15	60.0	10	0	1	9

Table 6. Observed vs. expected number of regressor- and progressor-type tumors formed in birds of the $B^{12}B^{15}$ and B^1B^{12} genotypes

B Genotype	Hatch	Type of Tumor and No.		Total N_T	χ^2	Prob.
		Regressor Obs - Exp[a]	Progressor Obs - Exp			
$B^{12}B^1$	1	21 - 19.1	2 - 3.9	23	0.60	.25<p<.50
(R-Rs-12,[b] r-Rs-1)	2	7 - 7.5	2 - 1.5	9	0.00	p → 1.
	Combined	28 - 26.6	4 - 5.4	32	0.18	.5<p<.75
$B^{12}B^{15}$	1	7 - 9.1	4 - 1.9	11	1.63	.10<p<.25
(R-Rs-12,[b] r-Rs-15)	2	8 - 11.6	6 - 2.1	14	4.83	.025<p<.05
	Combined	15 - 20.75	10 - 4.25	25	7.82	.005<p<.01

[a]Observed - Expected values.

Expected number of progressors = qi qj Nij.

Expected number of regressors Nij = (1 - qiqj) Nij.

Where:

qi - calculated frequency of recessive allele (progressor) in B^iB^i genotype

qj - calculated frequency of recessive allele (progressor) in B^jB^j genotype

q - is estimated as $\sqrt{Np/Nt}$ and Np - is number of progressor tumors in genotypes B^iB^i and B^jB^j.
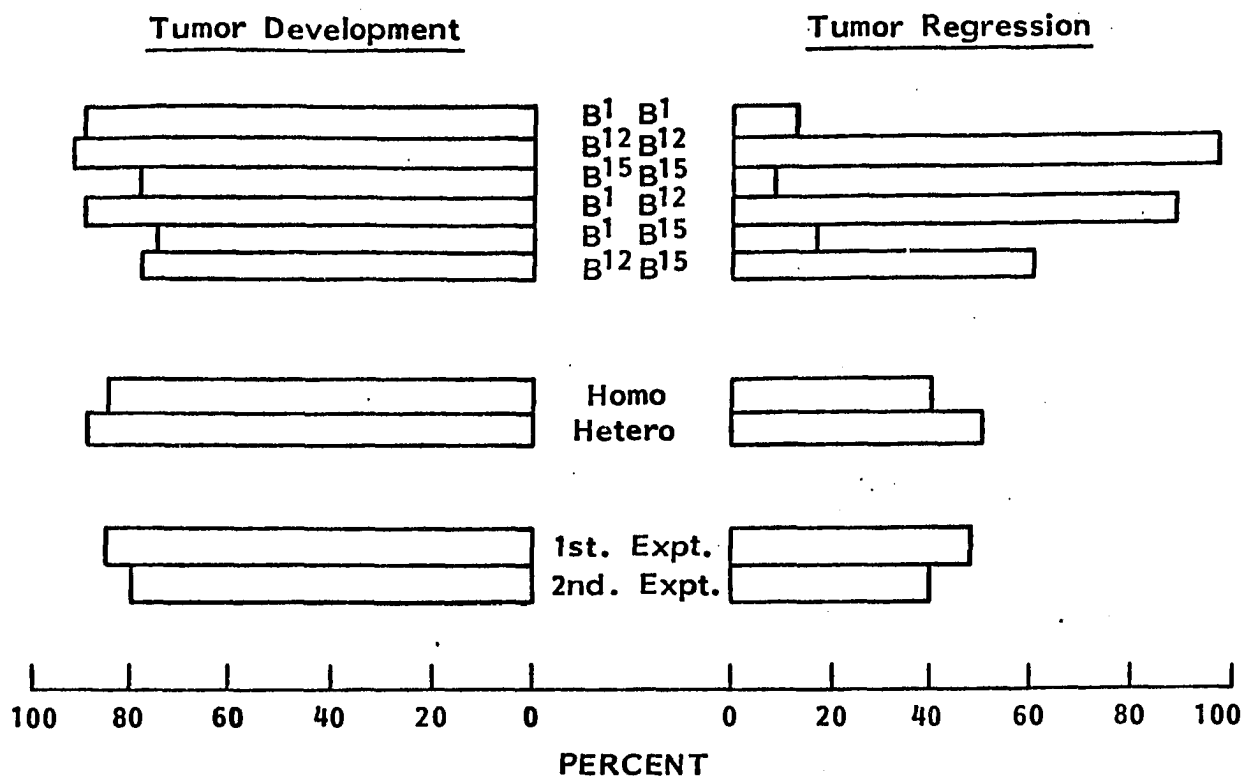
[b]Putative alleles.

Figure 1. Tumor development and tumor regression from wing web inoculation of RSV-A in HN inbred line

Single Versus Multiple Loci

Linked to the B Complex

The results are presented in Table 7. The level of regression ranged from zero and 100%. Part of this variation, no doubt, is the result of incomplete gene penetrance and (or) other variable conditions of virus administration or individual bird variation not associated with genotype.

Table 8 presents a χ^2 analysis for both cellular resistance to tumor development (Tv, tv genes) and for tumor regression (R-Rs, r-Rs genes) between TSV subgroups A, B, and C (2 degrees of freedom). The results clearly demonstrate that for the Tv, tv loci, which are not linked to the B complex, differences between virus subgroups in tumor formation are statistically significant in all cases at 0.01 level except for the $B^{13}B^{13}$ genotype. In contrast, for the R-Rs, r-Rs locus controlling tumor regression, only 3 of 10 B complexes showed statistical significance. These were for B^1B^1, $B^{13}B^{13}$, and $B^{21.1}B^{21.1}$. As already pointed out, the phenotypic level of tumor expression is probably dependent on total gene background. Also, because the results of each B complex represent an average over a variable number of lines, genetic background is not accurately controlled. Note that for combined data, differences between virus subgroups were not significant. Thus, the evidence suggests that a single locus hypothesis of tumor regression explains the variation in the data reasonably well. On the other hand, the fact that some of the individual χ^2 tests are statistically significant, leaves open the alternative possibility that loci linked to the B complex but unique to each RSV virus subgroup, control variation in tumor regression.

Returning to Table 7, we divided the scale of percentage regressors

into 3 arbitrary classes: less than 25%, progressor (P); 25 to 75%, progressor/regressor (P/R); more than 75%, regressor (R). This gave the following distribution:

	RSV-A	RSV-B	RSV-C
B^1B^1	P	P	P
B^6B^6	-	R	R
$B^{12}B^{12}$	R	R	R
$B^{13}B^{13}$	P	P/R	P/R
$B^{15.1}B^{15.1}$	P/R	P/R	P/R
$B^{21.1}B^{21.1}$	P/R	-	P
B^YB^Y	P/R	-	P/R
B^ZB^Z	R	-	R
WR	P	P	P/R
WS	P/R	P	P/R

The results indicate that the B^1 haplotype behaves as a progressor for all 3 virus subgroups, B^6 behaves as a regressor for B and C (A not tested), B^{12} behaves as a regressor for all 3, B^{13} is questionable but probably a progressor, $B^{15.1}$ is intermediate for all 3, $B^{21.1}$ is questionable, but probably a progressor, B^Y is intermediate on A and C, B^Z is a regressor on A anc C, WR is probably a progressor, and WS is questionable but probably intermediate. Consistent results between RSV subgroups are evidence for a single locus hypothesis as in the case of B^1B^1 and $B^{12}B^{12}$. Inconsistent results are evidence to reject the single locus hypothesis.

Although, this analysis of the results is inconclusive, it seems that these are insufficient evidence to reject the single-locus hypothesis. The

classification of intermediate, or P/R, suggests that the observed tumor phenotype for a given B complex is dependent on total interacting background genes in the total genome. Also the possibility exists that an intermediate classification denotes multiple alleles.

Table 7. Incidence of tumor expression induced by wing web inoculation of RSV subgroups A, B, and C

B Blood Genotype	RSV-A			RSV-B			RSV-C		
	nf/N[a,b]	nr/nt[c,d]	Reg.[e] %	nf/N	nr/nt	Reg. %	nf/N	nr/nt	Reg. %
B^1B^1	11/78	13/67	19.0	39/69	7/30	23.0	20/69	1/49	2.0
B^6B^6	28/28	–	–	0/15	14/15	93.3	16/18	2/2	100.0
$B^{12}B^{12}$	1/16	14/15	93.3	3/15	10/12	83.3	9/14	5/5	100.0
$B^{13}B^{13}$	28/86	9/58	15.5	27/52	9/25	36.0	15/53	17/38	44.7
$B^{15.1}B^{15.1}$	28/86	32/73	43.8	51/96	20/45	44.4	39/93	21/54	38.9
$B^{21.1}B^{21.1}$	2/26	15/24	62.5	27/27	–	–	0/20	2/20	10.0
B^YB^Y	3/26	14/23	60.9	11/11	–	–	1/18	7/17	41.2
B^ZB^Z	2/18	16/16	100.0	20/20	–	–	12/25	10/13	76.9
WR	12/16	1/4	25.0	15/16	0/1	0.0	7/16	7/16	33.3
WS	10/15	2/5	40.0	14/16	0/2	0.0	7/22	5/15	33.3

[a] nf = number giving a negative response.

[b] N = total number inoculated.

[c] nr = number of regressors.

[d] nt = number with tumors.

[e] % Reg. = regressors.

Table 8. Chi-squared tests for cellular resistance (Tv, tv genes) and
for tumor regression (R-Rs, r-Rs genes) between RSV
subgroups A, B, and C (wing web inoculation)

	Tv, tv expression		Rs expression	
	χ^2	d.f.	χ^2	d.f.
$_B1_B1$	26.9**	2	9.4**	2
$_B6_B6$	52.2**	2	0.1	1
$_B{12}_B{12}$	13.1**	2	1.4	2
$_B{13}_B{13}$	7.4	2	10.3**	2
$_B{15.1}_B{15.1}$	13.2**	2	0.4	2
$_B{21.1}_B{21.1}$	65.3**	2	10.3**	1
$_BY_BY$	36.9**	2	0.8	1
$_BZ_BZ$	30.7**	2	3.0	1
WR	9.9**	2	0.5	2
WS	12.4**	2	1.1	2
Combined Data	76.9**	2	6.7	2

**$P \leq 0.01$.

Role of B Complex Genotype in Cellular

Resistance and Tumor Regression Mechanisms

Cellular resistant (tv) loci

Gene frequencies at the tv loci, estimated from both the CAM and WWC

tests, are presented in Table 9 for the different inbred lines grouped

according to B blood types in common. Based on the CAM test (Table 9A),

differences in tv gene frequencies varied from 0 to 0.97. Chi square

tests for line differences with B blood types in common were significant

at $P \leq 0.01$ for all 3 virus subgroups.

Turning to the WWC test (Table 9B), estimates of gene frequencies

ranged from 0 to 1.0. Part of this variation is, no doubt, a consequence

of incomplete penetrance, small numbers, and sampling errors. With the

exception of lines within B^1B^1 blood type for RSV-A, the χ^2 differences for

lines with common B blood types were significant at $P \leq 0.01$ for all 3

subgroup viruses.

Differences in cellular resistance (CR) among inbred lines ignoring

blood types (IL/BT), were contrasted with differences among B blood types

ignoring lines (BT/IL). For the CAM test (Figures 2 and 3), differences

in CR among IL/BT was greater than among BT/IL. The range was 0 to 94.7%

for the former and 0.38 to 0.89 for the latter. IL/BT differences were

statistically significant for all 3 virus subgroups. Conversely, BT/IL

were not statistically significant for subgroups A and C, although B was

significant at the 5% level.

The most highly resistant lines were GHS for RSV-subgroup A; lines 8,

9, SP, and M for subgroup B; and lines 8, 9, and GHS for subgroup C. All

other lines were either susceptible or apparently were still segregating. These results support the studies reported by Collins et al. (1980) that the tv loci in general, maps outside the B complex.

Reference to Figures (2 and 3) shows differences in CR ranging from 0 to 1.0 for IL/BT but only from 0.38 to 0.84 for BT/IL. Statistical differences for the former were highly significant for all 3 virus subgroups but none were significant for the latter. The tv results from the WWC test were in good agreement with those from the CAM test.

Correlations of tv gene expression for 2-way combinations for virus subgroups A, B, and C are presented in Table 10. The statistical evidence showing that tvc and tva loci are linked confirms the earlier findings of Dren and Pani (1977).

Tumor regression (rs) loci

Estimates of gene frequencies (p') for lines with common blood types IL/BT are given in Table 11. With one exception, χ^2 values are not statistically significant. This implies that genetic control, generally, does not reside outside the B complex. The exception is the group with the B^1B^1 blood types in common challenged with RSV-A (χ^2 = 25.00, d.f. = 5). In this case, the Fayoumi line M with 59% regressors (10/17) contrasts with the remaining pool of 5 lines of which 47 individuals are progressors and only 3 are regressors (6%). Thus, we have evidence that at least part of the genetic variance in tumore regression in the Fayoumi line may be controlled by genes outside the B complex.

The contrast of IL/BT in tumor regression, versus that between BT/IL is shown in Figure 4. The highest percentages of tumor regressors were in

lines 8, 9, and M and the lowest were in lines 19 and HN for RSV-A. In addition the $B^{15.1}$ allele had the highest percentage and the B^1 allele had the lowest percentage for tumor regression for subgroups A anc D. Therefore, our results generally confirm those presented by Schierman et al. (1977) and Collins et al. (1977) that rs genes are linked to the B complex.

Table 12 compares the magnitude of line variance (σ_L^2) within the same B blood types, versus the variance of the mean B blood types, (σ_B^2) within the same lines for both the tv and rs loci. This permits a statistical measure of the relative importance of non-B versus B-linked genetic differences. The ratios, σ_L^2/σ_B^2, were 3.2, 17.3, and 0.2 for the tv loci (CAM test), tv loci (WWC test), and for the rs loci (WW test), respectively. Thus, line variances were 3.2- and 17.3-fold larger than blood type variances based on the CAM and WWC tests for cellular resistance, respectively. In contrast, blood type variance was 5-fold larger than line variance for tumor regression.

Correlation coefficients between the CAM and WWC tests, as calculated from the incidence of positive (or negative) reactions to total tests is given in Table 12. As expected, the correlations of the two tests for cellular resistance are high and positive ranging from 0.65 to 0.89. In contrast, the correlations of incidence of cellular resistance to incidence of tumor regression are lower and negative except for subgroup A (r = 0.42 and 0.12). These results support the two-locus hypothesis of resistance although cellular resistance and tumor regression are not wholly independent events. That is, they are gene frequency dependent. As an extreme example, differences in tumor regression can only be shown in birds which lack cellular resistance.

The "tv" results of this study support the hypothesis that cellular resistance to tumor viruses is controlled by single pairs of autosomal genes, that susceptibility is dominant to resistance, and that tva genes are linked to tvc but not to tvb in agreement with Pani and Biggs (1974). In addition, the results confirm those of Collins et al. (1977) and Schierman et al. (1977) that primary control of tumor regression rests with "rs" genes mapping in the B complex but that non-B complex rs genes may cooperate in tumor regression (Collins et al., 1980).

An important question in the development and use of inbred lines relates to the residual heterozygosity after inbreeding. The expected probability of gene segregation (Q) decreases with inbreeding but also it is a function of the gene frequency in the line before inbreeding. In particular,

$$Q = 2pq \ (1 - Ft) \tag{1}$$

where p and q are the allelic frequencies (p = q = 1), and Ft is Wright's inbreeding coefficient in generation t (Hartl, 1980). For example, consider line M with the lowest calculated inbreeding coefficient (F = 0.81) of 8 lines (Table 1). The probability of segregation at locus tva is $Q = 2(.5)(.5)(1 - .8) = 0.9$ assuming p = 0.5 = q. Conversely the probability of fixation is $1 - Q = 1 - 0.9 = 0.1$.

At the other extreme, line HN (Table 1) with the highest calculated inbreeding (F = 0.97), the expected Q at locus tva is .015. Thus, we expect the theoretical probability of heterozygosity remaining among the 8 partially inbred lines in Table 1 for a given locus is in the approximate range of 1.5 to 10%.

Furthermore, the number of lines all segregating at a given locus

should be binomially distributed assuming the lines have independent

origin. Consider n lines of which r are segregating and n-r are fixed at,

say, locus tva. For any one line, P is the probability of fixation and Q

is the probability of segregation. Then the probability of n-r lines

being fixed and r lines segregating at locus tva is $CrP^{n-r}Q^r$, where

$Cr = n!/(n-r)!r!$

For example, suppose we have 8 lines each with inbreeding coefficients

of 80%; assume initial allelic frequencies at tva are P = 0.5 = q. Then

substitution in formula (1) gives P = 0.9 and Q = 0.1. Expansion of the

binomial $(P + Q)^n$ generates the following probabilities:

Term	Cr	$P^{n-r}Q^r$			Prob.	
P^8	1	x	$.9^8$		.43	
P^7Q	8	x	$.9^7$	x 10^{-1}	.38	
P^6Q^2	28	x	$.9^6$	x 10^{-2}	.15	
P^5Q^3	56	x	$.9^5$	x 10^{-3}	.03	
P^4Q^4	70	x	$.9^4$	x 10^{-4}	.005	.995
P^3Q^5	56	x	$.9^3$	x 10^{-5}	.000	
P^2Q^6	28	x	$.9^2$	x 10^{-6}	.000	
$P Q^7$	8	x	$.9$	x 10^{-7}	.000	
Q^8	1	x		10^{-8}	.000	.005
						1.000

Thus, the probability that all 8 lines are fixed at the same locus,

as tva, is 0.43; the probability that only 1 line of 8 is segregating at

tva is 0.38; that 2 lines are segregating is 0.15 and the probability that

no more than 4 lines are segregating at tva is 0.995.

For three loci, as tva, tvb, and tvc, the theoretical problem is more

complex but straightforward if the three loci are known to be independent or if their linkages are known.

A perusal of the results reported here would suggest that the observed apparent segregation at both the tv and rs loci is greater than one would expect with coefficients of inbreeding in the observed range of our values 0.81 to 0.97. We, therefore, deduce that forces exist which retard the expected progress towards gene fixation.

Abplanalp (in Altman and Katz, 1979) has discussed some of these forces and has pointed out that the chance for pedigree errors is considerably greater in chickens than in mice. This comes about because a mouse litter naturally identifies with its mother, but with chickens, using modern artificial hatching and rearing, this cannot happen. In chickens, a single male is usually pen-mated to several hens; this may lead to errors in marking eggs, misreading numbers, chick mix-ups on hatching, and possibly other kinds of clerical errors.

An important tool to minimize such errors and to maintain genetic purity is blood typing. Because this practice has been followed for the past several years in our laboratory, we believe we have been able to avoid pedigree errors. Therefore, we believe factors other than pedigree errors account for the maintenance of seemingly excessive heterozygosity in some of our lines. Of possible importance is natural selection and the greater fitness of the heterozygote. A second possibility is incomplete penetrance of a gene. Perhaps this rather vague concept is better interpreted in terms of nonuniform environmental effects. For example, two chickens of the same genotype may give different responses when treated with a given virus, because it is not possible to administer

exactly the same dose of virus particles to each. Moreover, the response mechanisms of two different chickens would not be the same because the overall immune mechanisms of each would differ in subtle ways depending on prior encounters with ubiquitous random environment and disease.

This raises some question of the validity of estimation and use of allelic frequencies in data of this type. If penetrance of a gene is incomplete, or if its expression is influenced by nonuniform environmental effects, then gene frequency estimates may contain a bias. For example, if some genetically susceptible genotypes don't express the challenge expected from a dose of virus, they then would be misclassified as resistant. In a given test, let

S = fraction of those tested which are truly susceptible

R = fraction of those tested which are truly resistant

a = fraction of susceptible genotypes not responding to challenge.

An extimate of the allelic frequency of, say, the tva gene would be (assuming resistant allele is recessive):

$$q(tva) = \sqrt{R + a}$$

It follows that the estimated frequency p, of the allele Tva, is $1 - \sqrt{R + a}$. Thus, when a > 0, q(tv) is biased upwards and p(tv) is biased downwards. Supposing that in a highly inbred line S = .9, R = 0, and a = 0.1. Then, the estimated $q(tv) = \sqrt{0 + 0.1}$ = .32 and p(Tv) - 1 - .32 = .68. Thus, incomplete penetrance or environmental effects, can seriously bias estimates of gene frequency. As a consequence, this situation will make it appear that segregation is greater than expected in a highly inbred line.

This suggests that an analysis of the genetic basis of resistance to, say, RSV infection requires a quantitative approach.

Pani and Biggs (1974) estimated the heritability of genes controlling

susceptibility of the chick embryo CAM to RSV infection. Their data,

consisting of two parental lines, plus an Fl and an F2 population,

clearly demonstrated a single pair of alleles controlling CAM foci

development. One parent line was susceptible and the other resistant.

Heritability was estimated as,

$$h^2 = \frac{VF2 - \sqrt{VP1 \cdot VP2}}{VF2} = 0.71$$

where the V's are variances of the F2, Pl, and P2 (parental) populations.

This estimate is in close agreement with an earlier study reported by

Bower et al. (1964) of $h^2 = 0.72$.

Genetic variance is partitionable into additive and nonadditive

fractions. When dominance is complete, i.e., the heterozygote is equal to

the homozygous dominant, then the total genetic variance is partitionable

into additive (σA^2) and dominance deviation (σD^2) fractions. Thus,

$h^2 = (\sigma A^2 + \sigma D^2)/(\sigma A^2 + \sigma D^2 + \sigma E^2)$ is referred to as heritability in the

broad sense (Lush, 1948). We deduce that $\sigma E^2 = 1 - h^2 = 0.30$ is the

estimated fraction of nongenetic variance associated with a CAM test, and

perhaps also the WW test, for RSV susceptibility; accordingly, it serves

as part of the explanation as to why several of the partially inbred lines

tested in this study appear to be segregating for both tv and rs loci

greater than expected as judged by their inbreeding coefficients.

The estimate of $h^2 = 0.71$, as reported by Pani and Biggs (1974), is

maximal because the allelic frequencies in the F2 population are equal

to $p = 0.5 = q$. Thus, whenever allele frequencies differ, i.e., $p \neq q$,

the genetic variance would be less than maximum. The general expression

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is, $(\sigma A^2 + \sigma D^2) = 2pq[a + (q - p)d]^2 + (2pqd)^2$ where the first term is

the additive fraction and the second the dominance fraction of genetic

variance (Hartl, 1980). The "a" is the average effect of the gene substi-

tution and d is the dominance effect. When dominance is complete, a = d.

If a is set equal to 1, then $\sigma A^2 = \frac{1}{2}$ and $\sigma D^2 = \frac{1}{4}$. In this case, the

fraction of genetic variance, $\sigma A^2 + \sigma D^2$, is equal to 3/4 of the total.

This is maximal, as one might expect, in an F2 population. On the other

hand, if gene frequencies are, say, p = 0.1 and q = 0.9, then $\sigma A^2 + \sigma D^2 =$

.58 + .03 = .61. Conversely, if p = 0.9 and q = 0.1, then $\sigma A^2 = 0.1$ and

$\sigma D^2 = .03$.

Obviously, when p or q = 1, then the total genetic variance is zero.

On the other hand, the nongenetic fraction of variance, σe^2, earlier

alluded to, is generally assumed not to be influenced by changes in gene

frequency. Thus, based on the Pani and Biggs (1974) study, we deduce that

about 30% of the variance in our estimates of resistance/susceptibility is

of nongenetic origin within the lines studied.

Table 9a. Chi squared test for significance of cellular resistance (tv) among inbred lines with common blood types to three Rous sarcoma virus subgroups. Data from CAM test

	RSV - A[a]		RSV - B		RSV - C	
Line	nf/N	q(tv)	nf/N	q(tv)	nf/N	q(tv)

Blood type B^1B^1

8	2/10	0.45	9/10	0.95	8/12	0.82
19	2/25	0.28	2/20	0.32	1/15	0.26
GH	0/11	0.00	5/11	0.67	1/11	0.30
HN	1/11	0.30	1/10	0.32	6/11	0.74
SP	0/10	0.00	7/11	0.80	0/10	0.00
M	7/16	0.66	10/14	0.85	1/10	0.32

χ^2 for 5 d.f.   16.15[**]   28.34[**]   24.98[**]

Blood type $B^{15.1}B^{15.1}$

8	14/25	0.75	22/24	0.96	10/13	0.88
9	9/20	0.67	18/19	0.97	10/14	0.85
19	1/13	0.28	9/12	0.87	3/10	0.55
GH	5/28	0.42	17/29	0.77	5/19	0.51

χ^2 for 3 d.f.   13.88[**]   12.36[**]   12.20[**]

Blood type $B^{13}B^{13}$

19	0/22	0.00	1/11	0.30	4/10	0.63
GH	1/21	0.22	11/18	0.78	0/14	0.00
GHs	17/21	0.90	0/18	0.00	12/17	0.84

χ^2 for 2 d.f.   34.32[**]   19.70[**]   16.24[**]

[a]Fraction of cellular resistant embryos to total number inoculated, $\sqrt{nf/N}$ = q(tv).

[**]$P \leq 0.01$.

Table 9b. Chi squared test for significance of cellular resistance (tv) among inbred lines with common blood types to three Rous sarcoma virus subgroups. Data from Wing Web challenge

Line	RSV-A[a]		RSV - B		RSV - C	
	nf/N	q(tv)	nf/N	q(tv)	nf/N	q(tv)
Blood type B^1B^1						
8	1/ 6	0.41	5/ 5	1.00	4/ 4	1.00
19	6/23	0.51	10/20	0.71	0/16	0.00
GH	0/15	0.00	6/16	0.61	1/12	0.29
HN	1/10	0.32	1/10	0.32	8/17	0.69
SP	0/ 4	0.00	5/ 6	0.91	0/ 7	0.00
M	3/20	0.39	10/10	1.00	7/13	0.73

χ^2 for 5 d.f.   6.18   24.42[**]   27.69[**]

Blood type $B^{15.1}B^{15.1}$

Line	nf/N	q(tv)	nf/N	q(tv)	nf/N	q(tv)
8	6/16	0.61	15/15	1.00	16/16	1.00
9	16/24	0.82	21/21	1.00	18/18	1.00
19	1/19	0.23	7/17	0.64	0/17	0.00
GH	1/20	0.20	8/20	0.63	0/20	0.00

χ^2 for 3 d.f.   27.11[**]   30.75[**]   70.89[**]

Blood type $B^{13}B^{13}$

Line	nf/N	q(tv)	nf/N	q(tv)	nf/N	q(tv)
19	0/29	0.00	4/15	0.52	0/18	0.00
GH	0/29	0.00	16/20	0.89	2/19	0.32
GHs	28/28	1.00	7/17	0.64	13/16	0.90

χ^2 for 2 d.f.   86.05[**]   10.92[**]   32.44[**]

[a]Fraction of cellular resistant birds to total number inoculated, $\sqrt{nf/N}$ = q(tv).

[**]$P \leq 0.01$.

Table 10. Correlations of tv gene expression for 2-way combinations of virus subgroups

	CAM	WWC	Combined
Correlation d.f. $\longrightarrow$	6	6	12
rAB	−.07	.21	.09
rAC	.59	$.71^{*}$	$.69^{**}$
rBC	−.03	.53	.37

*P < 0.05.

**P < 0.01.

Table 11. Chi squared test for significance in tumor regression (rs) among inbred lines with blood types in common to three RSV subgroups. Data from Wing Web challenge

Line	RSV - A[a] np/nt	p(rs)	RSV - B np/nt	p(rs)	RSV - C np/nt	p(rs)
Blood type B^1B^1						
8	4/ 5	0.11	–	–	–	–
19	17/17	0.00	9/10	0.05	16/16	0.00
GH	15/15	0.00	7/10	0.16	11/11	0.00
HN	8/ 9	0.06	6/ 9	0.18	7/ 7	0.06
SP	3/ 4	0.13	1/ 1	0.00	6/ 6	0.00
M	7/17	0.36	–	–	6/ 6	0.00
x^2, (d.f.)	25.00**	(5)	2.01	(3)	4.63	(4)
Blood type $B^{15.1}B^{15.1}$						
8	4/10	0.37	–	–	–	–
9	4/ 8	0.29	–	–	–	–
19	12/18	0.18	2/10	0.55	9/17	0.27
GH	6/19	0.44	3/12	0.50	8/20	0.37
x^2, (d.f.)	4.84	(3)	0.09	(1)	0.63	(1)
Blood type $B^{13}B^{13}$						
19	24/29	0.09	7/11	0.20	12/18	0.18
GH	25/29	0.07	2/ 4	0.29	7/17	0.36
GHs	–	–	7/10	0.16	2/ 3	0.18
x^2, (d.f.)	0.14	(1)	0.56	(2)	2.48	(2)

[a]Fraction of number of progressors to total number with tumors, $\sqrt{np/nt}$ - p.

**$P \leq 0.01$.

Table 12. Comparison of line variance (σ_L^2) within blood types versus variance of mean blood types (σ_B^2) within lines for incidence of cellular resistance (tv) and tumor regression (rs)

	RSV-subgroups			Blood type	RSV-subgroups			σ_L^2 among lines / σ_B^2 among B's
Line	A	B	C		A	B	C	
cellular resistance (CAM test)[a]								
8	0.46	0.91	0.72	B^1	0.09	0.39	0.26	
19	0.05	0.28	0.23	$B^{15.1}$	0.30	0.74	0.43	
GH	0.10	0.57	0.14	B^{13}	0.02	0.41	0.17	
$\bar{X}$	0.20	0.59	0.36	$\bar{X}$	0.14	0.51	0.29	
$\sigma_L^2 = 0.0617$				$\sigma_B^2 = 0.0193$				3.2
cellular resistance (Wing Web Test)[b]								
8	0.32	1.00	1.00	B^1	0.16	0.51	0.16	
19	0.10	0.40	0.00	$B^{15.1}$	0.16	0.58	0.30	
GH	0.02	0.54	0.06	B^{13}	0.15	0.58	0.30	
$\bar{X}$	0.15	0.65	0.35	$\bar{X}$	0.10	0.55	0.17	
$\sigma_L^2 = 0.1093$				$\sigma_B^2 = 0.0063$				17.3
tumor regression (Wing Web Test)[c]								
8	0.53	–	–	B^1	0.97	0.80	1.00	
19	0.83	0.58	0.73	$B^{15.1}$	0.47	0.23	0.46	
GH	0.73	0.46	0.54	B^{13}	0.84	0.60	0.54	
$\bar{X}$	0.70	0.52	0.64	$\bar{X}$	0.76	0.54	0.67	
$\sigma_L^2 = 0.0120$				$\sigma_B^2 = 0.0590$				0.20

[a]Fraction of cellular resistant embryos to total number inoculated.
[b]Fraction of cellular resistant birds to total number inoculated.
[c]Fraction of number of progressors to total number with tumor.

Table 13. Correlations between the CAM tests and the WWC test for
           expressions of cellular resistance and tumor regression

Correlation	RSV-subgroups		
	A	B	C
r12	0.89^{**}	0.70^{**}	0.65^{*}
r13	0.42	-0.06	-0.15
r23	0.12	-0.53	-0.43

Test

1   CAM, cellular resistance

2   WW, cellular resistance

3   WW, tumor regression

$^{*}P < 0.05$, d.f. = 11.

$^{**}P < 0.01$.
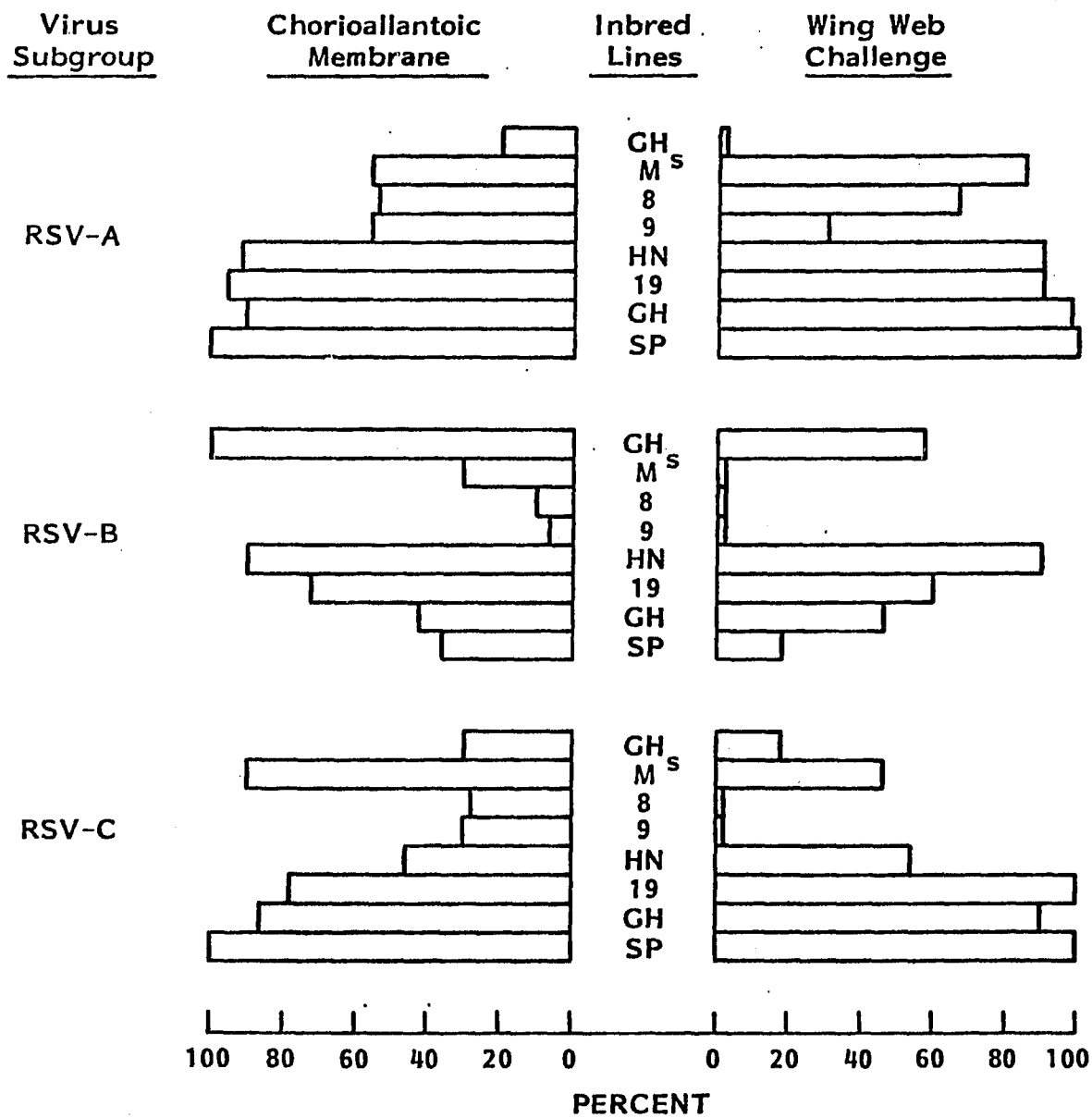
Figure 2. Tumor development of different inbred lines ignoring

B blood types at 70 day post-inoculation

Virus Subgroup	Chorioallantoic Membrane	Blood Type	Wing Web Challenge
RSV-A		B^1 B^{13} B^{15}	
RSV-B		B^1 B^{13} B^{15}	
RSV-C		B^1 B^{13} B^{15}	

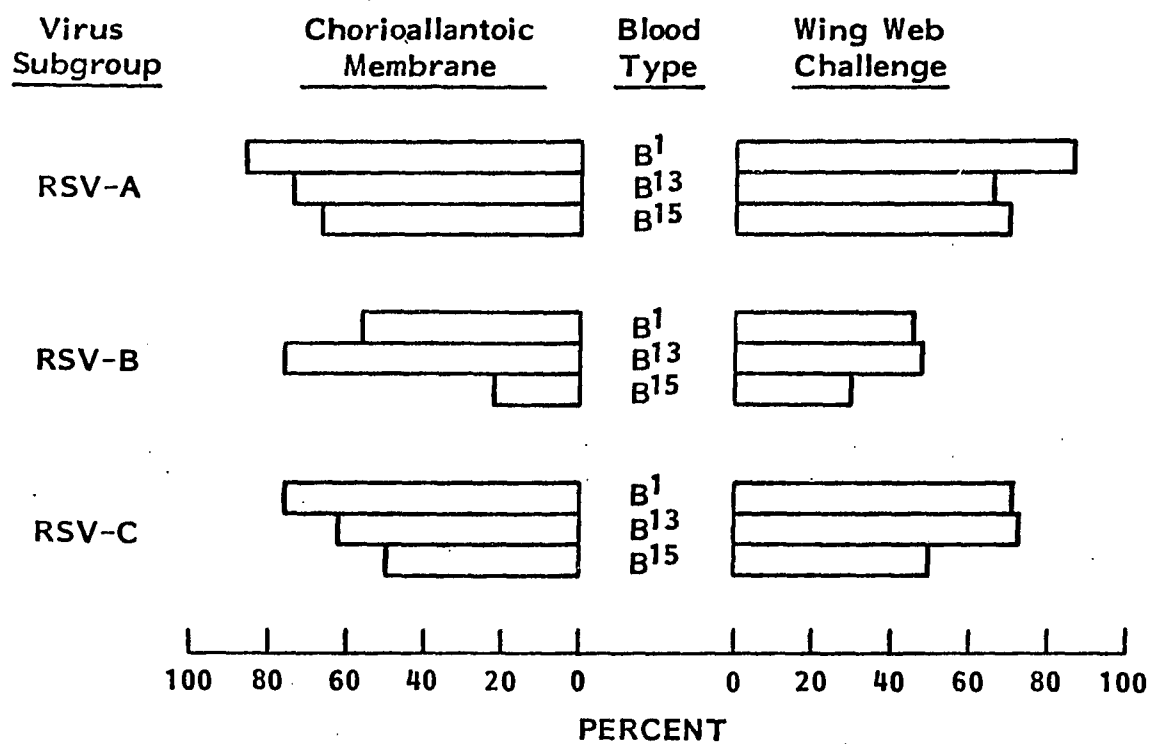100 80 60 40 20 0   0 20 40 60 80 100

PERCENT

Figure 3. Tumor development of different B blood types ignoring lines at 70 days post-inoculation
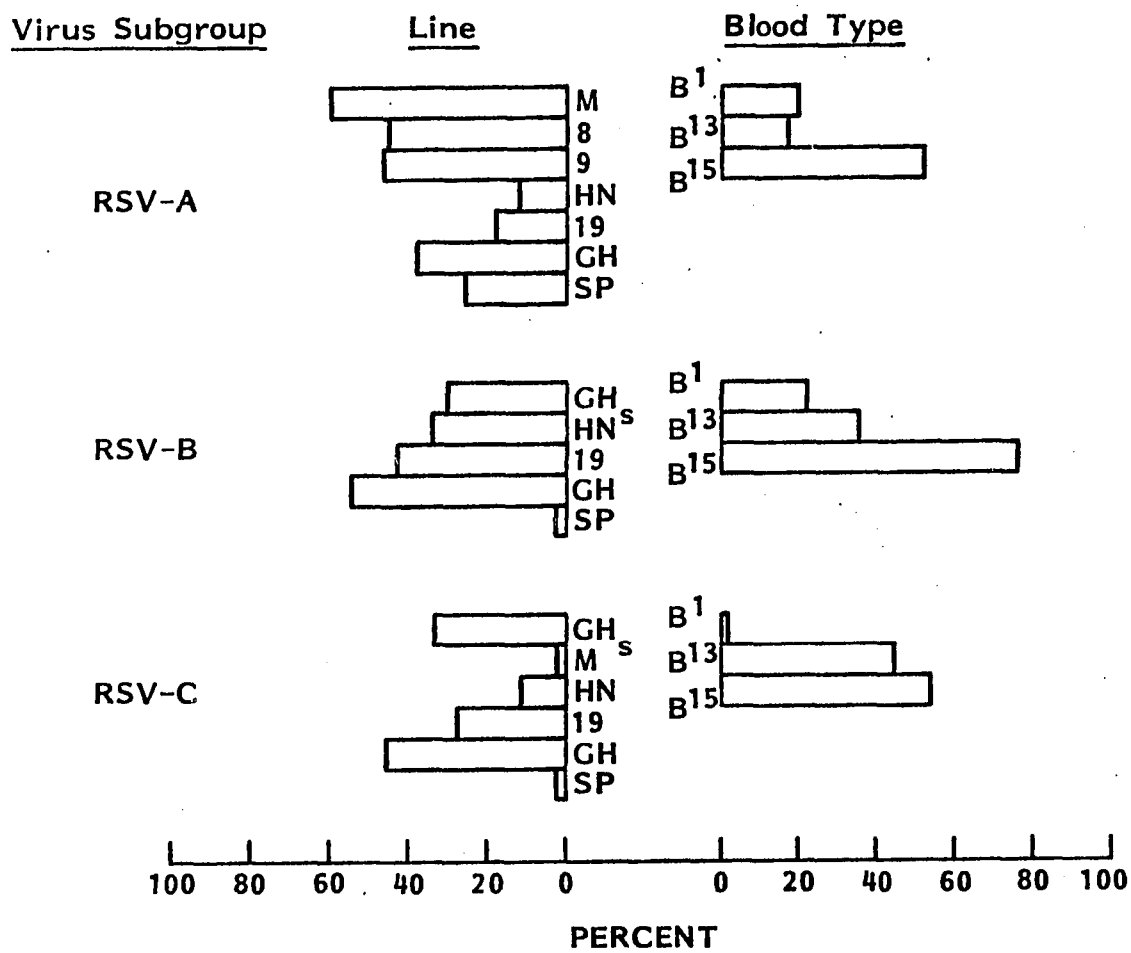
Figure 4. Tumor regression at 70 days post-inoculation between inbred lines ignoring blood types, versus that between blood types ignoring lines

GENETIC LINKAGE BETWEEN IMMUNE RESPONSE TO GAT

AND THE FATE OF RSV-INDUCED TUMORS IN CHICKENS

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ABSTRACT

Recent studies suggest that the gene locus controlling the fate of tumors induced by Rous sarcoma virus (RSV) is linked to the B histocompatibility complex. Birds carrying the dominant allele regress the tumor; homozygous recessives being unable to do so, develop large tumors and die. These are called progressors.

The Bryan strain of RSV was inoculated into 220 6 week old Leghorns homozygous for B^1B^1, B^2B^2, or $B^{19}B^{19}$ of which the percentages of progressors were 79, 22 and 56, respectively. The balance of each were regressors and survived.

The B^1B^1 test birds were derived from special matings, i.e., high and low immune responders to the amino acid polymer, GAT. Of 67 tests progeny of the B^1B^1 GAT-low mating, 63 or 94% proved to be progressors, and 6% were regressors. Of 84 test progeny of the B^1B^1 GAT-high matings, 67% were progressors, and 33% were regressors. The difference between the high and low GAT responders is highly significant and indicates that the locus controlling the fate of RSV-induced tumors is closely linked to the locus controlling immune response to GAT. The latter maps within the Ir region of the B histocompatibility complex.

INTRODUCTION

Previous studies have reported evidence for genetic control of resistance and susceptibility to infection frou Rous sarcoma virus (RSV) inoculation in young chickens (Waters and Burmester 1961, Crittenden et al. 1964, Gyles et al. 1968). More recently Collins and co-workers (1977) and Schierman and co-workers (1977) demonstrated that genetic control of the fate of RSV tumors is closely linked to, or within, the B major histocompatibility complex. In addition, the Schierman group found that regression of RSV tumors, induced by the Schmidt-Ruppin strain of subgroup B, is determined by a dominant gene designated R-Rs-1[1]. The recessive allele, designated r-Rs-1[1], permits uncontrolled (progressive) tumor growth in homozygous birds. Our study, reported herein, indicates that the RSV genes map within the Ir region of the B complex. In particular, we have found close linkage between the RSV genes, which control tumor regression to subgroup A RSV, and the immune response locus (Ir-GAT), which controls antibody production to the amino acid polymer, GAT^{10} (Pevzner et al. 1978).

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[1]For convenience we use Rs and rs to designate resistant and susceptible alleles, respectively.

MATERIALS AND METHODS

Birds used in this study were S1 line Leghorns homozygous for alleles B^1, B^2 and B^{19} (Nordskog et al. 1973). A subpopulation of the S1 line was subjected to 2-way selection for high and low antibody titer after inoculation with Salmonella pullorum bacterin (Pevzner et al. 1977). Within each genotype, B^1B^1, B^2B^2, and $B^{19}B^{19}$, divergent selection for S. pullorum titer was continued over four successive generations. In the second generation, breeders were tested for immune response to GAT. The fourth generation progeny of the B^1B^1 genotype came from four mating types: High-High, High-Low, Low-High and Low-Low with respect to GAT response and to S. pullorum titer. In addition, B^2B^2 and $B^{19}B^{19}$ birds testing, respectively, as intermediate and as high responders to GAT, were included in the experiment. Divergent selection for S. pullorum titer within these genotypes did not prove successful. Thus, essentially all B^2B^2 and $B^{19}B^{19}$ birds used in this study were high (or "normal") responders to S. pullorum.

Rous sarcoma virus (RSV) identified as avian RNA leukosis-sarcoma subgroup A RSV-1 Bryan strain, was kindly supplied by Dr. L. B. Crittenden, RPRL, East Lansing, Michigan. A total of 220 birds were inoculated in the subcutaneous tissue of the left wing web at 6 weeks of age with a 26-gauge 3/4 in. needle. A dosage of 0.05 ml per chick with 1:200 dilution was used.

All birds were examined once each week from the 7th to the 70th day post-inoculation.

Tumor responses induced by RSV were scored for size on a scale ranging from 0 (no response) to 6 (massive tumor) following essentially the same procedure as given by Collins and co-workers (1977). Those developing from day 7 to 28, 29 to 49 and 50 to 70 days were arbitrarily designated

as early, intermediate and late periods, respectively,

It will be desirable to make a clear distinction between "haplotype" and "genotype" as used in this paper. The former defines constitution of the gamete with respect to three loci which are believed to control (a) serological determinants (as the B blood group), (b) immune response to GAT, and (c) RSV-induced tumor response. "Genotype" will designate either the haplotypic composition of the diploid individual or, less specifically, only the serological blood type of a bird, as B^1B^1.

RESULTS

All birds tested gave an initially positive tumor response. These were classified as "regressor" or "progressor" in accordance with classification used by others (Gyles et al. 1968, Schierman et al. 1977, Collins et al. 1977). Birds of three blood types, B^1B^1, B^2B^2 and $B^{19}B^{19}$ were classified into six genotypes on the basis of their immune response to GAT and their serum antibody titer to S. pullorum. Of a grand total of 220 birds inoculated, 75 (34%) were classed as regressors, and 145 (66%) as progressors. Differences between the average results for the six genotypes (Table 1) were highly significant statistically. Average differences between the B genotypes and between the high-low immune response-to-GAT genotypes were highly significant, but were not between the high and low titer-to-S. pullorum genotypes.

Table 1 also summarizes the tumor responses within each of the three B genotypes. The B^1B^1 had the lowest percentage of regressors: 21.2% were regressors, and 78.8% were progressors. For the B^2B^2 genotypes, the percentages were almost exactly reversed. In general, the B^2B^2 birds were strong regressors, and the $B^{19}B^{19}$ were intermediate with about 44% being regressor and 66% progressor. Differences were statistically significant (P < 0.01).

Table 2 summarizes the tumor response from RSV virus inoculation into B^1B^1 birds, exclusively. The first part contrasts the GAT-low and GAT-high groups. Thirty-three percent of the latter, but only 6% of the former, were regressors. Correspondingly, the percentages of progressors were 67.0% and 94.0%. The difference is highly significant.

Table 2 also presents the contrast between the progeny of high and

low titer parents to S. pullorum. Differences were small and not statis-
tically significant. The conclusion is that the (RS,rs) locus controlling
the fate of the RSV induced tumors, is closely linked to the Ir-GAT
locus and therefore maps within or close to the Ir region of the B
complex.

Three arbitrary post-inoculation periods of tumor development were
scored for average tumor size; this is presented in Figure 1 for the B^1B^1
birds of both GAT-low responder parents and of GAT-high responder parents.
For the regressor-type tumors, the GAT-high responders develop tumors most
rapidly. On the other hand, tumor development was slower and more uniform
throughout the test period for the GAT-low group. For the progressive-
type tumors, most of the GAT-low responders showed rapid development and
those of the GAT-high responders, slower development.

Scores for tumor development (Fig. 1B) averaged around 2 for the
regressor-type and between 5 and 6 for the progressor-type. Differences
between periods and between GAT responders were small and not statistically
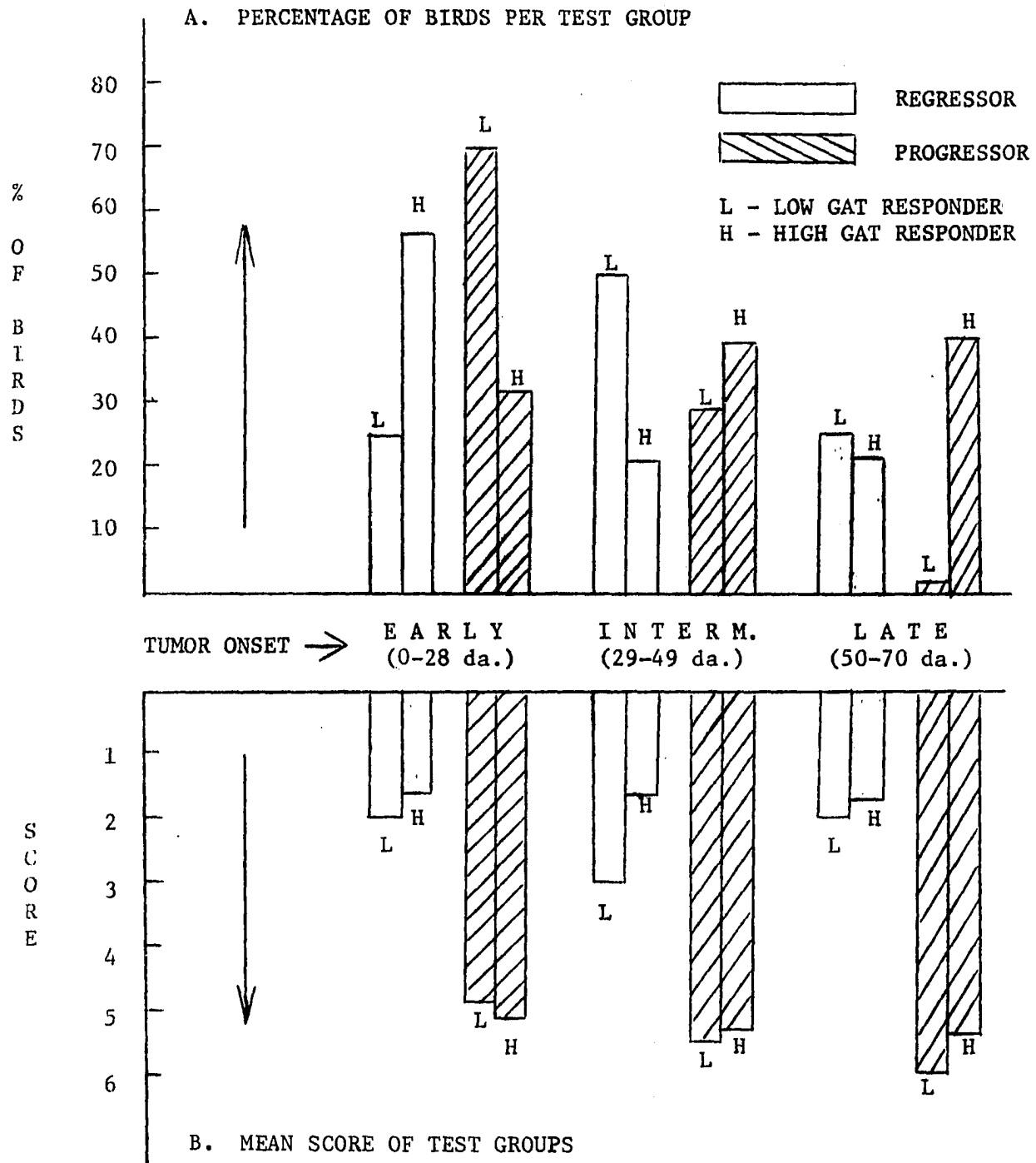significant.

Fig. 1A and B.   Tumor development classified by subgroups and initial period of development: Early (0-28 days), Intermediate (29-49) days and Late (50-70 days).   Percentage of birds is given in (A) and average score is given in (B)

DISCUSSION

The genetic mechanism involved in resistance to the leukosis/sarcoma viruses can be described in terms of a first and second line of defense (Crittenden 1975). The first is cellular resistance, i.e., a mechanism that prevents a virus from penetrating living host cells. Cellular resistance is genetically controlled by single genes specific to each virus subtype. Thus, the tva locus controls resistance to subgroup A viruses with susceptibility being completely dominant to resistance. The second line of defense is a mechanism of genetic resistance to tumor development which comes into play after the virus has already penetrated the cell. The resistance mechanism seems to interfere in the reproduction of viral progeny such that the host develops an immunity which leads to, say, tumor regression.

Collins and co-workers (1977) and Schierman and co-workers (1977) reported that regression of tumors induced by RSV A and B, respectively, is controlled by genes in the B complex. Because all the chickens inoculated with RSV A virus in our study developed tumors, this indicates that they lacked cellular resistance.

Genes coding for control of the fate of Rous sarcoma virus induced tumors are evidently closely linked to the Ir-GAT locus. Whether the former maps within the Ir region is indeterminate or until a marker gene is found which would define the limit of the Ir region in one direction (as Ss-Slp in mice). For purposes of discussion, we assume the following linear order of regions and genes on the chromosome encompassing the B major histocompatibility complex:

Region	SD			Ir		

Genes	B	Ir-GAT		Rs,rs
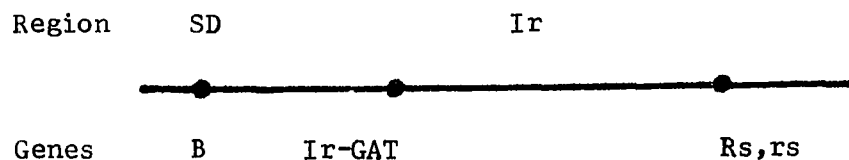
The SD region may consist of two loci, as in the human HLA system, but in chickens this is not yet fully demonstrated. The Ir region includes the Ir-GAT locus and possibly also (Rs,rs).

Birds classified as homozygous B^1 genotype, based solely on the criteria of serological typing, may contain any pair-combination of the four haplotypes:

					Frequency in mating:	
No.		Haplotype		Origin	GAT-low	GAT-high
I	B^1	Ir(G-L)	rs	parental	P	P
II	B^1	Ir(G-L)	Rs	crossover	Q	q
III	B^1	Ir(G-H)	rs	crossover	-	r
IV	B^1	Ir(G-H)	Rs	"parental"	-	s

Break point   a   b   P + Q = 1

$$p + q + r + s = 1$$

Haplotype I is assumed to be the typical or predominant haplotype origina originally found in the B^1B^1 blood groups of our S1 line. Assuming that haplotype IV was derived as a crossover at break point a from matings of B^1B^{19} heterozygotes, i.e.,

$$\frac{B^1 \qquad\qquad Ir(G\text{-}L) \qquad\qquad rs}{B^{19} \qquad\qquad Ir(G\text{-}H) \qquad\qquad Rs}$$, we hypothesize the formation

of haplotypes II and III. Such recombinants involving both B^1 and B^{19} are likely to have occurred with low frequency; those with superior fitness qualities may have accumulated over the many years of special matings in the S1 line (Nordskog et al. 1973) at the expense of those with low fitness through natural selection.

The frequencies of the four haplotypes in the two special matings of the S1 population (Table 2) depends on the amount of chromosome breakage at b, which in turn depends on the tightness of linkage between the two adjacent loci as well as on the composition of the particular sample of breeders selected for each mating.

A unique feature of the data is that GAT response was measured only in the parental generation and RSV tumor response was measured only in the progeny generation. This was not only a matter of expedience but also it seemed unwise to test for GAT response in the progeny as this might interfere with the tests for tumor response. At the same time, this experimental design does not invalidate the estimation of gene frequencies (and haplotype frequencies) by application of the Hardy-Weinberg equilibrium law (Falconer 1960. A special theorem of the Hardy-Weinberg law is that gene frequency equilibrium is completely reached in the first progeny generation irrespective of the parental genotypic (or haplotypic) composition. Furthermore, the expected gene frequencies in the progeny will be the same as that in the selected sample of parental breeders.

Frequency of Haplotypes of GAT-low Matings

Only haplotypes I and II would be involved in GAT-low matings assuming that Ir(G-L) is recessive to Ir(G-H). The frequency of haplotype I in the test progeny is then estimable from application of the Hardy-Weinberg principle. From Table 2 we set $P = \sqrt{.94} = .97$ and $Q = 1 - P = .03$. Thus, we deduce, that the frequency of the parental haplotype I in the GAT-low matings is 97% and that of the crossover haplotype, II, is 3%. This hypothesis accounts for the 6% of regressor progeny found in the GAT-low matings.

Frequency of Haplotypes in the GAT-high Matings

Breeders in the GAT-high matings could consist of any combination of the four haplotypes except I/I, I/II or II/II. Because haplotype IV is recently derived and the frequency of crossover haplotypes II and III should be low, most of the breeders in the GAT-high matings are expected to be the heterozygote, I/IV.

From Table 2 the frequency of progressors would consist of

$$P^2:I, \quad I+2pr:I, \quad III+r^2:III, \quad III=.667$$

Assuming that the frequency of crossover haplotype III is the same as for crossover haplotype II, we can solve for p,

$$r=Q=.03 \text{ and } Q=q.$$

Thus, $p^2 + 2p(.03) + (.03)^2 = .667$ and $p = .79$, the frequency of haplotype I.

For haplotype IV,

$$s = 1 - :79 - .03 - .03 = .15.$$

In summary, based on the presented, the estimated haplotypic frequencies of the progeny of the GAT-low and GAT-high matings are as follows:

| Mating | Tested progeny N freq. | Frequency of haplotypes | | | | |
		I p	II q	III r	IV s	Total
GAT-low	67	.97	.03	–	–	1.00
GAT-high	84	.79	.03	.03	.15	1.00
Combined	151	.87	.03	.02	.08	1.00

From the combined estimates based on a weighted average of the two separate mating groups, and applying the Hardy-Weinberg law, we predict that the overall mean percentage of progressors is $100p^2 = 100(.87 \pm .03)^2$ = 81%. This compares with the observed percentages (Table 2) of 78.3 and 79.4, respectively, for the S. pull.-low and S. pull.-high groups and fully accounts for the nonsignificant effect of pullorom antibody titer on the fate of RSV-induced tumors.

In addition to the four haplotypes proposed for the B^1B^1 blood group in our S1 population, we hypothesize a "parental" haplotype

$$\underline{B^{19} \quad \underset{a}{\quad} Ir(G-H) \quad \underset{b}{\quad} Rs}$$ for the $B^{19}B^{19}$ blood group with possible recombinant haplotypes resulting from crossovers at a and b. Likewise, for out B^2B^2 blood group, the typical haplotypes is tentatively defined as $B^2 Ir(G-INTERMEDIATE)Rs$. The GAT allele in the Ir region is probably different from the GAT-Low and GAT-High alleles common to the B^1 and B^{19} blood groups.

Map Distance

The estimate of 3% for each crossover haplotype II and III, should not be construed as an estimate of map distance between the GAT locus and (Rs, rs). Rather, the 3% is presumed to represent the net accumulation of crossover haplotypes over a period of many years. Crossing over has been

enhanced by deliberately maintaining forced heterozygosity of B blood

groups over 10 or more years in the Sl line (Nordskog et al. 1973, 1977).

Furthermore, we assume that the MHC in the Sl line is in linkage disequi-

librium. Thus, the accumulation of recombination haplotypes should

continue each successive generation but at a progressively slower rate

until linkage equilibrium is reached. A crude estimate of map distance

between GAT and (Rs,rs) is .03/10 $\cong$ .3 of 1% crossover units. Thus, it

would seem reasonable to speculate that (Rs,rs) is located in the Ir

region of the MHC.

Table 1. Tumor response from inoculation of RSV in six different B complex genotypes

B complex	No. inoculated	Negative	Regressor No.	%	Progressor No.	%
B^1B^1 GAT-low S.P.-low	37	0	1	2.7	36	97.3
B^1B^1 GAT-low S.P.-high	30	0	3	10.0	27	90.0
B^1B^1 GAT-high S.P.-low	46	0	17	37.0	29	63.0
B^1B^1 GAT-high S.P.-high	38	0	11	29.0	27	71.0
B^2B^2 GAT-intermediate	37	0	29	78.4	8	21.6
$B^{19}B^{19}$ GAT-high	32	0	14	43.8	18	56.2

$\chi^2 = 58.19$ d.f. $= 5$ $P \leq 0.01$

Summary by serotypes:

	No. inoculated		Regressor No.	%	Progressor No.	%
B^1B^1	151		32	21.2	119	78.8
B^2B^2	37		29	78.4	8	21.6
$B^{19}B^{19}$	32		14	43.8	18	56.2
	220		75		145	

$\chi^2 = 44.50$ d.f. $= 2$ $P \leq 0.01$

Table 2. Summary of tumor response from inoculation of RSV of B^1B^1 birds
classified according to immune response to GAT and to S. pullorum
titer

Classification of breeder population	No. Progeny Inoculated	Regressor		Progressor		χ^2	d.f.	P
		No.	%	No.	%			
GAT-low	67	4	6.0	63	94.0	16.71	1	$\leq$ 0.01
GAT-high	84	28	33.3	56	66.7			
	151	32		119				
S. pull.-low	83	18	21.7	65	78.3	0.025	1	N.S.
S. pull.-high	68	14	20.6	54	79.4			
	151	32		119				

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SUMMARY

The genetic mechanism for cellular resistance/susceptibility to tumor formation in chickens after challenge with Rous sarcoma virus (RSV) is known to be largely, if not entirely, independent of the B major histocompatibility complex. Moreover, specific loci control resistance to each virus subgroup, A, B, and C. However, C is closely linked to A but not to B. A distinctly separate genetic mechanism linked to the B complex controls tumor regression/progression. This mechanism comes into play for chickens which are genetically susceptible to tumor formation.

Tests were based on both wing web-challenge (WWC) on 5-week-old chicks and on the chorioallantioc membranes of 12-day embryos (CAM test). The absence of tumor development on WWC and of pock formation on CAM indicated cellular resistance to RSV (first line of genetic resistance). In addition, in WWC the differential response of tumors with respect to regressive or progressive tumor growth measured the second line of genetic resistance.

A comparison of tumor expression of the homozygotes, B^1B^1, $B^{12}B^{12}$, and $B^{15}B^{15}$ demonstrates that the genes linked to B^{12} and to B^1 behave essentially as a fully dominant/recessive allelic pair. On the other hand, the allelic pair corresponding to genes linked to B^{12} and B^{15} indicates that the former is incompletely dominant. Moreover, the two alleles, respectively, linked to the B^1 and B^{15} haplotypes, more or less recessive to the B^{12} linked allele, but differ distinctly one from the other. These results, therefore, indicate that the B complex-linked genes, controlling tumor expression, belong to a multiple allelic series at a single B complex

linked locus.

On addition, the data were not decisively conclusive with respect to a single locus or a multiple locus hypothesis but the former gives a reasonably satisfactory fit to the data. For the 10 genetic subgroups marked with different B locus blood groups, 3 gave statistically significant differences in tumor regression between virus subgroups and 7 did not. This is evidence for multiple loci. On the other hand, combining the results over the 10 groups, again with 2 degrees of freedom, the differences were not statistically significant. This is evidence for the alternative hypothesis of a single B complex-linked locus controlling tumor regression.

The results from both the WWC and CAM tests concluded that the tv loci for cellular resistance maps outside the B complex. In addition, the statistical evidence verifies the earlier findings that tva and tvc loci are linked. As expected, most of the variation in tumor regression in susceptible birds was linked to the B complex. An exception was the finding of significance between six inbred lines with the B^1B^1 blood type in common when challenged with RSV-A. Thus, part of the genetic variation in tumor regression may be controlled outside the B complex. In general, these results support the two locus hypothesis of resistance, although cellular resistance and tumor regression are not wholly independent events. Also, the results suggest that the observed apparent segregation at both the tv and rs loci is greater than expected in view of the high coefficients of inbreeding in our inbred lines. This is thought to be a consequence of natural selection and the greater fitness of the hetero-zygote and of incomplete gene penetrance.

Finally, the difference between the high and low GAT responders of B^1B^1 test birds is highly significant. It indicates that the locus controlling the fate of RSV-subgroup A induced tumors is closely linked to the locus controlling immune response to GAT and therefore maps within or close to the Ir region of the B complex.

GLOSSARY OF TERMS

Alloantigens: Different (allelic) forms of an antigen coded for at the same gene locus in all individuals of a species. E.g., histocompatibility antigens are coded for at the same locus but between individuals.

Alloantiserum: An antiserum directed against antigens of another animal of the same species and raised in that species. E.g., a serum made in one inbred strain of a species against another inbred strain of the same species.

B lymphocytes: Lymphocytes that are derived from bone marrow without passing through the thymus. In birds, B lymphocyte maturation is determined by the bursa of Fabricius. B lymphocytes play a major role in humoral immunity.

Bacterin: Vaccines consisting of suspension of bacterial cells that have been killed by chemical or physical means. E.g., Salmonella pullorum bacterin used as antigen in immune response reaction.

Blood group: Classification of isoantigens on the surfaces of erythrocytes. The most important blood group in chicken is B blood group system. This system now knows as the major histocompatibility complex or the B complex in the chicken.

CMI: Cell-mediated immunity, specific immunity which is dependent upon the presence of T lymphocytes. It is responsible for reactions such as allograft rejection, delayed hypersensitivity and is important in defense against viral infections.

Challenge: Administration of a virulent pathogen, e.g. in order to test

   initial immunity or the degree of protection achieved by

   treatment.

Congenic lines: Possessing identical genotypes except for a single

   difference. Those are useful for studies of genetic

   differences in the major histocompatibility complex.

GAT: Amino acid polymer, an antigen consists of L-glutamic acid^{60}, L-

   alanine^{30} and L-tyrosine^{10}. It is useful in immune response assay.

GVH: Graft-versus-host reaction, is a reaction of a graft rich in

   immunologically competent cells, against the tissues of a genetically

   non-identical recipient. The recipient is unable to reject the

   graft. It can be induced by immune immaturity, immunosuppression,

   or differences in genetic constitution (i.e., F_1 hybrid).

h^2: Heretability, a method used in selection to estimate the ratio of

   additive genetic variance to total phenotypic variance, VA/VP.

H-2: The major histocompatibility complex in the mouse. H-2 genes

   determine the major histocompatibility antigens on somatic cell

   surfaces and also the immune response of the animal (Ir genes). It

   is composed of five regions: K, I, S, D, and G.

H antigens: Histocompatibility antigens; the antigens responsible for

   tissue compatibility are designated as histocompatibility

   antigens and the genes coding for these structures are

   called histocompatibility genes (H genes).

Haplotype: Set of genetic determinants coded by closely linked genes on

   a single chromosome.

IR region: The immune response region of B-complex of chicken which is responsible for stimulating specific immune response against any foreign antigen.

IR gene(s): The immune response gene(s) located in immune response region of B-complex.

Inbred line: Experimental animals produced by sequential brother-sister matings. In immunology, the term usually refers to animals in the 20th and subsequent generations of such matings. Such animals are so homogeneous at histocompatibility loci.

Inoculation: In immunology refers to introduction of a substance into the body, usually but not exclusively by parenteral injection.

LLV: Lymphoid Leukosis virus; it is an avian, disease and is caused by an RNA myxo virus which affects almost any body tissue of a chicken producing tumors.

MD: Marek's disease; it is an avian disease and is caused by a DNA herpes virus typically leading to cancer of the nervous tissues.

MHC: Major histocompatibility complex; it codes for the cell membrane proteins which contain the major histocompatibility antigens. It is divisible into various regions on the basis of crossover analysis. Some species designations include: Ag-B in rat, B in chicken, H-z in mice and HLA in man.

MLR: Mixed lymphocyte reaction; mixed culture of lymphocytes from two donors and resulting in cellular proliferation if nonidentical at the MHc.

RSV: Rous sarcoma virus; it is a defective virus which is unable to
infect a cell except with the help of an Rous associated virus (RAV)
Because the host range of RSV coincides with that of LLV of the
same subgroup, RSV artificially-induced tumors using Rous sarcoma
virus is a useful model to study body defenses against oncogenecis.

SD: Serologically defined; blood cell antigens defined by the use of
specific antisera.

Titer: In serological reaction, a measure of the amount of antibody in
an antiserum per unit volume of original serum.

Tumor enhancement: An increased rate of tumor growth in animals immunized
with the antigens of the tumor of injected with dilution
of specific virus induced tumors.

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APPENDIX

Data from each inbred line have been listed
in the next tables.

Table 14. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line M.

B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
		No.	%	No.	%	No.	%
		RSV - A					
$B^y B^y$	26	3	11.5	14	53.9	9	34.6
$B^z B^z$	18	2	11.1	16	88.9	0	0.0
$B^1 B^1$	20	3	15.0	10	50.0	7	35.0
$B^1 B^y$	25	4	16.0	13	52.0	8	32.0
$B^1 B^z$	25	4	16.0	21	84.0	0	0.0
		RSV - B					
$B^y B^y$	11	11	100.0	0	0.0	0	0.0
$B^z B^z$	20	20	100.0	0	0.0	0	0.0
$B^1 B^1$	10	10	100.0	0	0.0	0	0.0
$B^1 B^y$	8	8	100.0	0	0.0	0	0.0
$B^1 B^z$	11	11	100.0	0	0.0	0	0.0
		RSV - C					
$B^y B^y$	18	1	5.6	7	38.9	10	55.5
$B^z B^z$	25	12	48.0	10	40.0	3	12.0
$B^1 B^1$	13	7	53.8	0	0.0	6	46.2
$B^1 B^y$	5	0	0.0	3	60.0	2	40.0
$B^1 B^z$	7	3	42.9	3	42.9	1	14.2

Table 15. Tumor response from inoculation of RSV-subgroups A, B and C of lines WR and WS

Line	Total No. Inoculated	Negative No.	%	Regressor No.	%	Progressor No.	%
		RSV - A					
WR	16	12	75.0	1	6.3	3	18.7
WS	15	10	66.7	2	13.3	3	20.0
		RSV - B					
WR	16	15	93.8	0	0.0	1	6.2
WS	16	14	87.5	0	0.0	2	12.5
		RSV - C					
WR	16	7	43.8	3	18.8	6	37.4
WS	22	7	31.8	5	22.7	10	45.5

Table 16. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of lines 8 and 9

Line	B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
			No.	%	No.	%	No.	%
		RSV - A						
9	$B^{15.1}B^{15.1}$	24	16	66.7	4	16.7	4	16.6
8	$B^{15.1}B^{15.1}$	16	6	37.5	6	37.5	4	25.0
	$B^{1}B^{1}$	6	1	16.7	1	16.7	4	66.6
		RSV - B						
9	$B^{15.1}B^{15.1}$	21	21	100.0	0	0.0	0	0.0
8	$B^{15.1}B^{15.1}$	15	15	100.0	0	0.0	0	0.0
	$B^{1}B^{1}$	5	5	100.0	0	0.0	0	0.0
		RSV - C						
9	$B^{15.1}B^{15.1}$	18	18	100.0	0	0.0	0	0.0
8	$B^{15.1}B^{15.1}$	16	16	100.0	0	0.0	0	0.0
	$B^{1}B^{1}$	4	4	100.0	0	0.0	0	0.0

Table 17. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line 19

B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
		No.	%	No.	%	No.	%
		RSV - A					
$B^{15.1}B^{15.1}$	19	1	5.3	6	31.6	12	63.1
$B^{13}B^{13}$	29	0	0.0	5	17.2	24	82.8
$B^{1}B^{1}$	23	6	26.1	0	0.0	17	73.9
$B^{13}B^{15.1}$	12	6	50.0	0	0.0	6	50.0
$B^{1}B^{13}$	14	2	14.3	1	7.1	11	78.6
		RSV - B					
$B^{15.1}B^{15.1}$	17	7	41.2	8	47.1	2	11.7
$B^{13}B^{13}$	15	4	26.7	4	26.7	7	46.5
$B^{1}B^{1}$	20	10	50.0	1	5.0	9	45.0
$B^{13}B^{15.1}$	12	4	33.3	5	41.7	3	25.0
$B^{1}B^{13}$	12	3	25.0	2	16.7	7	58.3

Table 17 continued.

B Complex Allele	Total No. Inoculated	Negative No.	%	Regressor No.	%	Progressor No.	%
				RSV - C			
$B^{15.1}B^{15.1}$	17	0	0.0	8	47.1	9	52.9
$B^{13}B^{13}$	18	0	0.0	6	33.3	12	66.7
B^1B^1	16	0	0.0	0	0.0	16	100.0
$B^{13}B^{15.1}$	12	0	0.0	4	33.3	8	66.7
B^1B^{13}	24	1	4.2	5	20.8	18	75.0

Table 18. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line GH

B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
		No.	%	No.	%	No.	%
RSV - A							
$B^{15.1}B^{15.1}$	20	1	5.0	13	65.0	6	30.0
$B^{13}B^{13}$	29	0	0.0	4	13.8	25	86.2
$B^{1}B^{1}$	15	0	0.0	0	0.0	15	100.0
RSV - B							
$B^{15.1}B^{15.1}$	20	8	40.0	9	45.0	3	15.0
$B^{13}B^{13}$	20	16	80.0	2	10.0	2	10.0
$B^{1}B^{1}$	16	6	37.5	3	18.8	7	43.7
RSV - C							
$B^{15.1}B^{15.1}$	20	0	0.0	12	60.0	8	40.0
$B^{13}B^{13}$	19	2	10.5	10	52.6	7	36.9
$B^{1}B^{1}$	12	1	8.3	0	0.0	11	91.7

Table 19. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line GHs

B Complex Allele	Total No. Inoculated	Negative No.	%	Regressor No.	%	Progressor No.	%
		RSV − A					
$B^{13}B^{13}$	28	28	100.0	0	0.0	0	0.0
$B^{6}B^{6}$	28	28	100.0	0	0.0	0	0.0
		RSV − B					
$B^{13}B^{13}$	17	7	41.2	3	17.6	7	41.2
$B^{6}B^{6}$	15	0	0.0	14	93.3	1	6.7
		RSV − C					
$B^{13}B^{13}$	16	13	81.2	1	6.3	2	12.5
$B^{6}B^{6}$	18	16	88.9	2	11.1	0	0.0

Table 20. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line HN

B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
		No.	%	No.	%	No.	%
			RSV - A				
$B^{15}B^{15}$	22	4	18.2	3	13.6	15	68.2
$B^{12}B^{12}$	16	1	6.3	14	87.5	1	6.2
$B^{1}B^{1}$	10	1	10.0	1	10.0	8	80.0
$B^{12}B^{15}$	14	3	21.4	7	50.0	4	28.6
$B^{1}B^{15}$	28	6	21.4	4	14.3	18	64.3
$B^{1}B^{12}$	25	2	8.0	21	84.0	2	8.0
			RSV - B				
$B^{15}B^{15}$	23	0	0.0	3	13.0	20	87.0
$B^{12}B^{12}$	15	3	20.0	10	66.7	2	13.3
$B^{1}B^{1}$	10	1	10.0	3	30.0	6	60.0
$B^{12}B^{15}$	6	2	33.3	4	66.7	0	0.0
$B^{1}B^{15}$	13	2	15.4	3	23.1	8	61.5
$B^{1}B^{12}$	8	0	0.0	7	87.5	1	12.5
			RSV - C				
$B^{15}B^{15}$	22	5	22.7	1	4.5	16	72.8
$B^{12}B^{12}$	14	9	64.3	5	35.7	0	0.0
$B^{1}B^{1}$	17	8	47.1	1	5.9	8	47.0
$B^{12}B^{15}$	6	2	33.3	3	50.0	1	16.7
$B^{1}B^{15}$	11	6	54.5	0	0.0	5	45.5
$B^{1}B^{12}$	6	3	50.0	3	50.0	0	0.0

Table 21. Tumor response from inoculation of RSV-subgroups A, B and C between B complex alleles of line SP

B Complex Allele	Total No. Inoculated	Negative		Regressor		Progressor	
		No.	%	No.	%	No.	%
		RSV - A					
$B^{21.1}B^{21.1}$	26	2	7.6	15	57.7	9	34.7
B^1B^1	4	0	0.0	1	25.0	3	75.0
$B^1B^{21.1}$	30	4	13.3	16	53.4	10	33.3
		RSV - B					
$B^{21.1}B^{21.1}$	27	27	100.0	0	0.0	0	0.0
B^1B^1	6	5	83.3	0	0.0	1	16.7
$B^1B^{21.1}$	15	14	93.3	1	6.7	0	0.0
		RSV - C					
$B^{21.1}B^{21.1}$	20	0	0.0	2	10.0	18	90.0
B^1B^1	7	0	0.0	0	0.0	7	100.0
$B^1B^{21.1}$	6	0	0.0	0	0.0	6	100.0

Table 22. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line GH

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
		RSV - A				
$B^{15.1}B^{15.1}$	28	17.9	14.3	7.1	14.3	46.4
$B^{13}B^{13}$	21	4.8	9.5	9.5	4.8	71.4
B^1B^1	11	0.0	0.0	0.0	9.1	90.9
		RSV - B				
$B^{15.1}B^{15.1}$	29	58.6	13.8	17.2	3.5	6.9
$B^{13}B^{13}$	18	61.1	11.1	16.7	5.6	5.5
B^1B^1	11	45.5	0.0	9.1	9.1	36.3
		RSV - C				
$B^{15.1}B^{15.1}$	19	26.3	10.5	10.5	15.8	36.9
$B^{13}B^{13}$	14	0.0	14.3	7.1	14.3	64.3
B^1B^1	11	9.1	9.1	0.0	9.1	72.7

Table 23. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line GHs

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
			RSV - A			
$B^{13}B^{13}$	21	81.0	14.3	4.7	0.0	0.0
B^6B^6	13	84.6	15.4	0.0	0.0	0.0
			RSV - B			
$B^{13}B^{13}$	18	0.0	0.0	11.1	22.2	66.7
B^6B^6	12	16.7	33.3	25.0	16.7	8.3
			RSV - C			
$B^{13}B^{13}$	17	70.6	5.8	11.8	0.0	11.8
B^6B^6	11	72.7	9.1	9.1	9.1	0.0

Table 24. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line SP

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
		RSV - A				
$B^{21.1}B^{21.1}$	22	27.3	31.8	4.6	13.6	22.7
B^1B^1	10	0.0	10.0	0.0	20.0	70.0
		RSV - B				
$B^{21.1}B^{21.1}$	20	85.0	5.0	10.0	0.0	0.0
B^1B^1	11	63.6	9.1	9.1	0.0	18.2
		RSV - C				
$B^{21.1}B^{21.1}$	14	0.0	7.1	7.2	21.4	64.3
B^1B^1	10	0.0	10.0	10.0	10.0	70.0

Table 25. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line HN

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
RSV - A						
$B^{15}B^{15}$	12	16.7	8.3	8.3	0.0	66.7
$B^{12}B^{12}$	15	53.3	26.7	13.3	6.7	0.0
$B^{1}B^{1}$	11	9.1	0.0	9.1	0.0	81.8
RSV - B						
$B^{15}B^{15}$	13	0.0	7.7	7.7	7.7	76.9
$B^{12}B^{12}$	12	25.0	8.3	33.3	16.7	16.7
$B^{1}B^{1}$	10	10.0	10.0	0.0	20.0	60.0
RSV - C						
$B^{15}B^{15}$	11	9.1	9.1	0.0	9.1	72.7
$B^{12}B^{12}$	13	61.5	15.4	7.7	7.7	7.7
$B^{1}B^{1}$	11	54.5	9.1	0.0	18.2	18.2

Table 26. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line M

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
			RSV - A			
B^yB^y	13	53.8	23.1	7.7	7.7	7.7
B^zB^z	19	84.2	15.8	0.0	0.0	0.0
B^1B^1	16	43.8	25.0	6.2	12.5	12.5
			RSV - B			
B^yB^y	13	69.2	30.8	0.0	0.0	0.0
B^zB^z	17	94.1	5.9	0.0	0.0	0.0
B^1B^1	14	71.4	21.4	7.2	0.0	0.0
			RSV - C			
B^yB^y	13	38.5	15.4	7.7	7.7	30.7
B^zB^z	12	50.0	8.3	16.7	8.3	16.7
B^1B^1	10	10.0	0.0	10.0	10.0	70.0

Table 27. Percentage of pock countoon chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C of lines WR and WS

Line	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
		RSV - A				
WR	16	93.8	6.2	0.0	0.0	0.0
WS	14	7.2	21.4	14.3	21.4	35.7
		RSV - B				
WR	28	64.3	32.1	3.6	0.0	0.0
WS	23	43.5	21.7	26.1	8.7	0.0
		RSV - C				
WR	13	76.9	15.4	7.7	0.0	0.0
WS	11	36.4	18.2	9.1	9.1	27.2

Table 28. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of line 19

B Complex Allele	No. Embryos	% Pock Counts				
		0	1-25	26-50	51-75	76+
				RSV - A		
$B^{15.1}B^{15.1}$	13	7.7	0.0	7.7	7.7	76.9
$B^{13}B^{13}$	22	0.0	4.5	4.5	9.1	81.9
$B^{1}B^{1}$	25	8.0	8.0	4.0	24.0	56.0
				RSV - B		
$B^{15.1}B^{15.1}$	12	75.0	16.7	8.3	0.0	0.0
$B^{13}B^{13}$	11	9.1	9.1	0.0	27.3	54.5
$B^{1}B^{1}$	20	10.0	15.0	10.0	5.0	60.0
				RSV - C		
$B^{15.1}B^{15.1}$	10	30.0	10.0	10.0	10.0	40.0
$B^{13}B^{13}$	10	40.0	10.0	10.0	20.0	20.0
$B^{1}B^{1}$	15	6.7	0.0	0.0	6.7	86.6

Table 29. Percentage of pock count on chorio-allantoic membranes (CAMs) from inoculation of RSV-subgroups A, B and C between B complex alleles of lines 8 and 9

Line	B Complex Allele	No. Embryos	% Pock Counts				
			0	1-25	26-50	51-75	76+
		RSV - A					
9	$B^{15.1}B^{15.1}$	20	45.0	15.0	10.0	10.0	20.0
8	$B^{15.1}B^{15.1}$	25	56.0	8.0	8.0	12.0	16.0
	B^1B^1	10	20.0	0.0	10.0	10.0	60.0
		RSV - B					
9	$B^{15.1}B^{15.1}$	19	94.7	5.3	0.0	0.0	0.0
8	$B^{15.1}B^{15.1}$	24	91.7	0.0	8.3	0.0	0.0
	B^1B^1	10	90.0	10.0	0.0	0.0	0.0
		RSV - C					
9	$B^{15.1}B^{15.1}$	14	71.4	21.5	7.1	0.0	0.0
-8	$B^{15.1}B^{15.1}$	13	76.9	15.4	7.7	0.0	0.0
	B^1B^1	12	66.7	16.7	8.3	8.3	0.0