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MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) VS NON-MHC INFLUENCES ON RESPONSE TO RSV-INDUCED TUMORS IN CHICKENS

DAVID WINSTON BROWN

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University of New Hampshire PH.D. 1982

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MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) VS. NON-MHC INFLUENCES ON RESPONSE TO RSV-INDUCED TUMORS IN CHICKENS

BY

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ABSTRACT

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) VS.
NON-MHC INFLUENCES ON RESPONSE TO
RSV-INDUCED TUMORS IN CHICKENS

by

DAVID W. BROWN

University of New Hampshire, September, 1982

The MHC of the chicken (B complex) has been shown to exert a decisive influence on the fate of Rous sarcoma virus (RSV)-induced tumors. However, noninbred chickens with identical B genotypes and inbred lines indistinguishable at the MHC have demonstrated considerable variation in response to RSV challenge. The major objective of this research was to determine the cause of variation in anti-Rous sarcoma response observed among chickens with "identical" B genotypes.

Chickens were evaluated for anti-tumor response following wingweb inoculation with RSV at six weeks of age. A comparison of anti-sarcoma responses of (15I-5 X 6-1)F1 versus (15I-5 X 7-2)F1 chickens revealed rapid regression among the (15I-5 X 6-1)F1's and tumor progression among the (15I-5 X 7-2)F1's. A three way cross between (15I-5 X 6-3)F1's and line 7-2 produced B2/B2 and B2/B15 progeny. B2/B15 chickens from this cross, identical at the B complex

to the (15I-5 X 7-2)F1 progressors, were characterized by tumor regression. Therefore a non-MHC influence associated with the line 7-2 background appeared to suppress anti-sarcoma response.

Similar findings were obtained using F2 and backcross progeny of lines 100 and UNH 105, low response being associated with the line 100 background. Both lines 7-2 and 100 are susceptible to Marek's disease (MD). Histological evidence for MD involvement was obtained from bursal sections of chickens that had died of progressive sarcoma growth. It is postulated that low anti-sarcoma response associated with MD susceptible lines may result from environmental exposure to MD and subsequent immunosuppression in spite of vaccination for MD at hatching. If low anti-sarcoma response associated with lines 100 and 7-2 is a result of immunosuppression by MD virus, then non-MHC genetic background would appear to be a strong factor influencing relative susceptibility to MD.

I. INTRODUCTION

A better understanding of the basic mechanisms involved in disease resistance can clearly aid in disease control and in the selection process for disease resistant plants and animals. Genes within the major histocompatibility complex (MHC), a tightly linked cluster of genes found in vertebrate species, exert a profound influence on host response to disease. Genetic studies dealing with the MHC of the mouse have proved of great value in providing insight into the mechanisms of the immune response of both man and animals.

A fairly consistent characteristic of the immune response has been its high degree of specificity. An MHC haplotype characterized by low response to a particular antigen can often respond well to other antigens. Because of such specificity, selection for resistance to one particular disease has the inherent danger of rendering a population susceptible to another disease or to a variant of the disease used for selection.

In natural populations of different species, the MHC has been found to be highly polymorphic. MHC polymorphism would clearly help protect a species from extermination by one of the many potential disease agents in the environment. Use of the MHC alone as a basis of selection for disease resistance could undermine such broad based protection by causing the loss of potentially valuable MHC haplotypes.

Knowledge of non-MHC genetic factors influencing resistance to specific diseases could prove useful in preventing such a loss.

The MHC of the chicken (B complex) has been shown to exert a decisive influence on the fate of RSV-induced sarcomas. Inbred chicken lines with indistinguishable (either serologically or by functional assays) B haplotypes, however, have varied considerably in their anti-Rous sarcoma responses. Indistinguishable is clearly not synonomous with identical, and the observed variation between lines in anti-tumor response could have resulted from either undetected variation within the MHC or to non-MHC influences (genetic and/or environmental). The major objective of the research discussed in this dissertation was to determine the cause of variation in anti-Rous sarcoma response observed among chickens with indistinguishable B genotypes.

II. REVIEW OF THE LITERATURE

The Major Histocompatibility Complex

The major histocompatibility complex (MHC) is a tightly linked cluster of genes responsible for various immunological phenomena including the regulation of cell-mediated and humoral immunity. Originally discovered in mice as the major cause of rapid rejection of incompatible neoplastic and normal tissue grafts (Counce et al. 1956), the MHC was subsequently found to play a major role in the regulation of immune responses to a wide variety of insults including synthetic polypeptides (McDevitt and Chinitz 1969, Benacerraf and McDevitt 1972) and more naturally encountered pathogens (Lilly 1968, Zinkernagel and Doherty 1975). The evolutionary significance of the MHC can be appreciated by fact of its presence in every mammalian species studied to date as well as its presence in non-mammalian vertebrates such as birds and bony fish (Klein 1977).

The MHC has been most thoroughly characterized in the mouse. Known as the H-2 complex in this species, it is currently divided into at least five regions - K, I, S, G, and D (Golub 1977, Benacerraf 1981). The K and D regions code for membrane bound glycoproteins found on nearly all cell surfaces but in highest concentrations on macrophages

and mature T and B lymphocytes (Klein 1977). These glycoproteins are the major stimulus for graft rejection and are key recognition elements involved in the intercellular cooperation required for generation of cytotoxic responses (Doherty et al. 1976). Arbitrarily designated as class I molecules, the products of the K and D regions are noncovalently associated with a smaller polypeptide, beta 2-microglobulin, encoded by a non-MHC gene (Klein 1977).

The I region codes for I associated (Ia) antigens. These glycoproteins, designated as class II antigens, are also found on cell surfaces. The distribution, however, is limited primarily to macrophages and B cells (Benacerraf 1981). Suppressor T cells bear antigens encoded by one particular subregion, I-J (Murphy et al. 1976), and soluble factors involved in regulation of immune response also bear Ia antigens (Benacerraf and Germain 1978). Immune response (Ir) genes, which control responses to a variety of thymus-dependent antigens, have been postulated to code for the Ia antigens. Alternatively, it has been suggested that Ir genes are primarily expressed on T cells and are involved in the production of specific helper and suppressor factors bearing determinants encoded by loci in the I-A and I-J subregions, respectively (Benacerraf and Unanue 1979). There is an increasing amount of evidence in support of the former hypothesis. Antisera directed against Ia antigens block T cell responses to antigens under Ir gene control (Schwartz et al. 1976). No recombinant animals have been

found that separate Ia loci and Ir loci from the same subregion of the I region (Fathman et al. 1981), and complementing Ir gene phenomena have been found to coincide with complementing Ia loci (Schwartz et al. 1978).

The G region codes for erythrocyte alloantigens (Schwartz 1980). Its functional significance has not been clearly determined. The S region codes for some components of the complement system (Carroll and Capra 1978). This system, consisting tentatively of 17 serum globulins, is involved in the humoral portion of the inflammatory response (Benacerraf and Unanue 1979). Sequential activation via a process of limited enzymatic proteolysis can lead to lysis of the target cell or, perhaps more importantly, to lymphokine production, chemotaxis of inflammatory cells, and enhanced opsonization.

The human MHC (HLA) is divided into four regions - D, B, C, and A. HLA-A, B, and C antigens correspond both structurally and functionally to the mouse K and D gene products (Benacerraf and Unanue 1979). HLA-D appears to be the human counterpart of H-2I of the mouse (Albert and Gotze 1977). In addition to the class I and II type antigens, several complement components (class III antigens) are associated with HLA (Benacerraf and Unanue 1979).

The B blood group system of the chicken was discovered by Briles et al. (1950) and shown to be a marker for the MHC by Schierman and Nordskog (1961). The B complex is located on a medium-sized microchromosome that also contains the

nucleolar organizer (Bloom et al 1978). It is currently divided into three regions, B-F, B-L, and B-G (Hala et al. 1977, Pink et al. 1977). Recombinants have been found which separate B-G from the B-F and B-L regions (Briles and Briles 1977, Hala et al. 1976), but recombinations between B-L and B-F have not been identified with certainty (Longenecker and Mosmann 1981).

The B-F region codes for surface antigens on most cells including leukocytes and erythrocytes. These glycoproteins are associated with beta 2-microglobulin-like molecules and may be analogous to the H2-K and H2-D products of mice (Ziegler and Pink 1975). B-L gene products may be analogous to Ia antigens of mice as judged by their occurrence exclusively on immunoglobulin-positive lymphocytes and monocytes/macrophages (Ewert and Cooper 1978, Crone et al. 1981). The B-G region codes for erythrocyte surface antigens which may be involved in differentiation of cells of the erythroid series (Longenecker and Mossman 1981). Although no immunological function has been attributed to the B-G region, Hala et al. (1981) recently reported that B-G antigen appeared to play a decisive role in the induction of humoral immunity against allogeneic B-F antigen on erythrocytes.

Like H-2 and HLA, the MHC of the chicken appears to influence serum complement levels (Chanh et al. 1976). Reports of immunological regulation associated with the B complex include responses to synthetic polypeptides (Gunther

et al. 1974, Benedict et al. 1975) and to virus-induced neoplasms (Longenecker et al. 1976, Briles et al. 1977, Collins et al. 1977, Schierman et al. 1977). The B complex has also been associated with autoimmune disease (Bacon and Rose 1979).

The RNA Tumor Viruses

History

In 1908 Ellerman and Bang reported the transmission of erythromyeloblastic leukemia of chickens by cell-free filtrates of leukemia cells (Tooze 1973). The first transmission of a solid tumor (a sarcoma of chickens) by cell-free filtrates was reported three years later by Rous (1911). Bittner (1936) obtained the first evidence that a mouse mammary gland carcinoma is transmitted from mother to offspring via a factor in the milk. This factor was later identified as a filterable virus (Andervont and Bryan 1944). Gross (1951) successfully transmitted leukemia to mice by inoculating with extracts of leukemia cells from a strain of mice with a high incidence of spontaneous leukemia. Since that time, several strains of murine leukemia viruses have been isolated, including the Friend, Moloney, Rauscher, and Graffi strains, and murine sarcoma viruses have also been identified (Tooze 1973). RNA tumor viruses have been incriminated as the cause of leukemias and/or sarcomas in a wide variety of different animal species including cats

(Jarrett et al. 1964), cattle (Kettman et al. 1976), and most recently, man (Lewin 1981, Robert-Guroff et al. 1982).

Pathology

Neoplasms are classified according to their tissue of origin. Carcinomas, for example, are malignant tumors of epithelial origin, whereas sarcomas are malignant tumors originating in connective tissue. Neoplasms of the hemopoietic and reticulo-endothelial systems may consist of solid masses of tumor cells or be characterized by leukemia, the circulation of large numbers of malignant cells. RNA tumor viruses are classified into three major groups: sarcoma, acute leukemia, and lymphoid leukosis viruses, on the basis of their pathogenicity in infected animals (Hanafusa 1981). Rous sarcoma virus causes rapid transformation of fibroblasts within 5-10 days after infection. Avian lymphoid leukosis viruses cause transformation of lymphoid cells of the bursa of Fabricius and have a latency period of several months. Acute leukemia viruses, in contrast, cause transformation of lymphoid cells of the bone marrow within 1-3 weeks after infection (Hanafusa 1981).

Mechanisms of transmission

Transmission of avian RNA tumor viruses can occur by several routes. Horizontal infection with leukosis viruses typically results in a transient viremia, immunity, and

rarely leukosis. Vertical transmission includes congenital infection and genetic transmission. Congenital infections are strictly maternal and result in chronic viremia and the frequent occurrence of leukosis (Rubin *et al.* 1962).

Both horizontal and congenital infections involve exogenous avian leukosis viruses (ALV's). Genetic transmission involves endogenous viruses which are integrated in the gametic DNA and are inherited as part of the chicken genome.

Relationship between avian sarcoma and leukosis viruses

The genomes of avian leukosis viruses contain three defined genes: *gag* coding for internal viral structural proteins, *pol* for the virion RNA-directed DNA polymerase (reverse transcriptase), and *env* for virion envelope glycoproteins (Coffin 1979). A fourth region, *c*, may contain promoter sequences (Robinson *et al.* 1980). The genetic structure of the lymphoid leukosis virus genome is in the order 5'-*gag*-*pol*-*env*-*c*-3'.

The avian sarcoma virus genome includes the *src* gene. The *src* gene product is a protein kinase with the unique ability to phosphorylate tyrosine residues of substrate proteins rather than serine or threonine residues, sites of phosphorylation for most known cellular protein kinases (Hunter and Sefton 1980). Since phosphorylation of proteins is associated with cell regulation, the increased level of phosphorylating activity by the *src* protein may trigger the

transformation process (Hanafusa 1981). Sequences in the normal avian genome have a high degree of homology with the *src* gene (Stehelin *et al*. 1976), and it is currently postulated that the sarcoma viruses as well as acute leukemia viruses arose from the recombinational events between leukosis viruses and the cellular genome of the host (Hanafusa 1981). Nondefective avian sarcoma viruses such as the Schmidt-Ruppin and Prague strains of Rous sarcoma virus (RSV) contain the entire genome of the avian leukosis viruses with the addition of the *src* gene inserted between *env* and *c* (5'-*gag*-*pol*-*env*-*src*-*c*-3'). Defective sarcoma viruses and the acute leukemia viruses are lacking portions of the ALV genome and require coinfection with an ALV for replication (Hanafusa 1981). In the case of the Bryan high titer strain of RSV (BH-RSV), the *env* gene is missing. *Env* gene products supplied by the coinfecting "Rous associated virus" (RAV) determine the subgroup specificity of the sarcoma virus resulting from such phenotypic mixing. Endogenous viruses which contain the *env* gene can also aid in the replication of the defective Bryan strain. Such endogenous *env* gene products were originally referred to as chick helper factor (chf) for their ability to aid in replication of BH-RSV in the absence of exogenous helper virus (Tooze 1973).

Cellular resistance to infection

Avian leukosis and sarcoma viruses have been classified

into subgroups on the basis of their host range in chickens. Susceptibility of a cell to infection appears to depend on the presence of receptor sites specific for viruses of each subgroup (Crittenden and Briles 1971). The plating efficiency of virus on resistant cells is 1/1000 or less the efficiency of plating on susceptible cells (Tooze 1973). Virus particles adsorb to both resistant and susceptible cells, but penetrate only susceptible ones (Crittenden 1968). Subgroup specificity is determined by the virus envelope glycoproteins (products of the *env* gene of leukosis or nondefective sarcoma viruses). Viruses of the same subgroup have the ability to interfere with each other, presumably by blocking the receptor sites. Leukosis virus-infected cells become resistant to infection with sarcoma viruses of the same subgroup, and such interference has been exploited as an assay of leukosis virus infection (Vogt and Ishizaki 1966).

Genetic Resistance to Avian Virus-Induced Tumors

Marek's disease

Hanson *et al.* (1967) suggested that the *B*21 haplotype conferred a higher level of resistance to Marek's disease (MD) than another *B* haplotype, *B*19. Pazderka *et al.* (1975) found that Cole's line N (Cole 1968), selected for resistance to MD, was uniformly homozygous for *B*21, while line P, selected in parallel for susceptibility, was

segregating for three or four other B haplotypes. The association of the B21 haplotype with resistance to MD was clearly demonstrated in experiments using F1 backcross (Briles et al. 1977) and F2 progenies (Longenecker et al. 1976) segregating at the MHC. Several B haplotypes have been more or less categorized according to degree of MD resistance. B21 exhibits strong resistance; B2 and B6, moderate resistance. B5, B13, and B15 are associated with high levels of susceptibility (Longenecker and Mosmann 1981).

Non-MHC influences on MD resistance have also been implicated. RPRL inbred lines 6 and 7 are homozygous for the B2 haplotype. Line 6 is resistant to MD, whereas line 7 is susceptible. The B haplotypes of the two lines are indistinguishable either serologically or by functional assays (Pazderka et al. 1975). Fredericksen et al. (1977) reported that Ly-4, a T-cell antigen of chickens, may influence relative susceptibility to MD. The different Ly-4 genotypes tested were produced from separate sets of non-inbred dams. Therefore other influences (both genetic and environmental) could have been the source of the observed variation.

Different isolates of MD virus vary greatly in their pathogenicity, both in terms of virulence and in their characteristic lesions (Powell 1981). Some highly virulent strains of MD virus have recently been described against which vaccination provides only partial protection (Witter

et al. 1980).

RSV-induced sarcomas

The B complex

Gyles and Brown (1971) demonstrated that the incidence of regression of RSV-induced sarcomas could be modified significantly by selection. Collins et al. (1977), using (6-1 X 15-1)F2 chickens segregating for three B genotypes, observed a strong influence of B genotype on ability to respond to BH-RSV(RAV-1) induced sarcomas. The percentages by genotype of a total of 376 B2/B2, B2/B5, and B5/B5 chickens dying of tumor by ten weeks postinoculation were 5, 26, and 93, respectively. The relative influences of sex, D genotype, and I genotype were considered insignificant since chi square values for the respective distributions of chickens to tumor profile index (TPI, an index of anti-tumor response) had associated probabilities greater than 0.01. However, all three calculated chi square values (i.e. for sex, D and I genotypes) had associated probabilities of less than 0.05. Subsequent hatches (Collins, unpublished data) indicated that the effects of sex and D genotype were insignificant. The I locus appeared to have a very subtle influence, if any.

The regressive potential associated with the B2 haplotype appeared to be strongly influenced by environmental conditions (Collins et al. 1977). One hatch totalling 109 chickens, not included among the 376 described

above, apparently was exposed to some undefined environmental insult which resulted in sporadic cases of "aplastic anemia". The percentages of these B2/B2, B2/B5, and B5/B5 chickens dying of tumor by ten weeks PI were 61, 77, and 97, respectively. Clearly an environmental variable existed which severely suppressed anti-tumor response.

B genotype appeared to influence the frequency of metastasis, since B5/B5 chickens which died of terminal tumors had a greater frequency of gross metastatic lesions (61%) than either B2/B2 (27%) or B2/B5 (23%) hosts (Collins et al. 1977). However, 80% of all B2/B2 and 55% of all B2/B5 chickens with terminal tumors were from hatches containing birds dying with aplastic anemia. The low incidence of metastasis associated with these two B genotypes may therefore have been indicative of another complicating factor which precipitated death prior to development of gross metastatic lesions.

Schierman et al. (1977) found a similar association between B genotype and ability to regress Schmidt-Ruppin (subgroup B) RSV-induced tumors using inbred lines G-B1 and G-B2 and crosses of these lines. G-B1 and G-B2 are homozygous for the B13 and B6 haplotypes, respectively (Briles and Briles 1981). Among 90 backcross progeny from F1 females mated to a G-B1 male, 96% of B13/B13 chickens developed progressive tumor growth compared to only 8% of the heterozygous B genotype B6/B13. Their interpretation of these results was that RSV-induced tumor regression was

controlled by a dominant gene for regression linked to the MHC. Results from progeny of B6/B13 heterozygous parents did not support such an interpretation, since nearly half of B6/B6 and B6/B13 progeny were progressors. Such results were tentatively attributed to possible recombination within the MHC, but non-MHC influences (genetic and/or environmental) could adequately and perhaps more plausibly explain the observed within genotype variation. No serological evidence was cited to indicate recombination.

Use of B complex recombinants has provided evidence that the B-F/B-L portion of the MHC is involved in regression of RSV-induced tumors (Collins and Briles 1981, Auclair et al. 1981).

Non-MHC influences

A possible non-MHC genetic influence on regression of RSV-induced tumors was reported by Marks et al. (1979). RPRL inbred lines 6-1, 6-3, and 7-2 (all homozygous for the B2 haplotype) were used to produce F1, F2, and reciprocal backcross progenies. Lines 6-1 and 6-3 were intermingled as a source of "line 6". Seventy-four % of (6 X 7-2)F1 X 7-2 backcross progeny were characterized by progressive tumor growth following challenge with BH-RSV(RAV-1). In contrast, only 6% of (6 X 7-2)F1 X 6 backcross progeny were progressors. (6 X 7-2)F2 chickens had a tumor progression incidence of 60%. A similar study (Marks et al. 1979) using BH-RSV(RAV-49) instead of BH-RSV(RAV-1) for challenge did not yield such dramatic differences. Reciprocal backcrosses

of (6-3 X 7-2)F1 chickens to line 6-3 were characterized by a 3% incidence of progressive tumor growth. Corresponding backcrosses to line 7-2 resulted in a 14% incidence of tumor progression. Although these results suggested non-MHC influences (either genetic, environmental, or both), they did not rule out a possible undetected difference between the B haplotypes of line 7-2 and lines 6-3 and 6-1.

Ly-4 and Th-1. Gilmour, Collins and others (manuscript submitted) have suggested that T lymphocyte alloantigens Ly-4 and Th-1 influence regression of Rous sarcomas. In one experiment using (6-3 X 7-2)F4 chickens, a Ly-4 X Th-1 interaction appeared to have a significant influence on response to BH-RSV(RAV-1)-induced tumors. Chickens having the Ly-4 and Th-1 genotypes characteristic of line 7-2 had a high frequency of regression. Thus Ly-4 and Th-1 influences would not explain the high incidence of progression reported by Marks et al. (1979). In a second experiment using (6-3 X 7-2)F5 chickens, Ly-4 appeared to significantly influence response and the Ly-4 X Th-1 interaction was not significant ($P > 0.05$). Again, chickens having the Ly-4 and Th-1 genotypes of line 7-2 had a high incidence of regression. In both experiments, separate sets of noninbred dams produced each of the Ly-4/Th-1 genotype combinations. Thus other influences (genetic and/or environmental) could have been major sources of variation. Assuming that F2, F4, and F5 generations of the cross would have similar incidences of tumor regression, the lower responses of (6 X 7-2)F2

chickens reported by Marks et al. (1979) could indicate an environmental variable capable of suppressing response.

The L locus. Collins (unpublished data) used (6-3 X 100)F2 chickens segregating at the C, D, E, I, and L alloantigen loci to investigate the possible influence of these loci on Rous sarcoma regression. The C, D, E, and I loci appeared to have no significant effect on response to RSV-1 induced sarcomas. The L locus appeared to have some influence on tumor regression in females. Some F1 dams were homozygous at the L locus resulting in unequal distribution of L genotypes to progeny of various dams. Such could have resulted in maternal influences on responses associated with different L genotypes. The relatively high incidence of progression among chickens containing the high responding B2 haplotype of line 6-3 and the presence of progeny resistant to tumor induction might suggest that some chickens were congenitally infected with avian leukosis virus. Progeny were not vaccinated for MD, so variations in maternal antibody could have been another important maternal influence.

Endogenous viruses. The endogenous avian leukosis viruses could conceivably influence susceptibility to neoplasia in at least two ways. They could be the actual agents of cell transformation, or they could tolerize the animal to cross reacting antigens associated with a closely related oncogenic exogenous virus. Endogenous ALV's, when introduced into chickens as exogenous infections, do not

appear to cause disease (Motta *et al*. 1975). However, recombination of a defective exogenous virus with an endogenous virus can result in an infective virus (Hanafusa *et al*. 1970). Robinson *et al*. (1980) demonstrated that recombinant viruses that contained the *gag*, *pol*, and *env* genes of endogenous viruses and the *c* region of exogenous viruses caused a similar incidence of disease as exogenous virus RAV-1. These findings suggested that the differences in oncogenic potential between endogenous and exogenous ALV's resided in the *c* region. It was postulated that the *c* region contained promoter sequences which, upon insertion into the host genome, caused expression of adjacent cellular genes as well as the viral genome. The failure of nondefective endogenous viruses to induce transformation may therefore reflect an inefficient endogenous promoter.

Halpern and Friis (1978) reported that endogenous viral genes influence the ability of the chicken to respond to the envelope glycoproteins of the related avian sarcoma virus. Chickens which are chick helper factor (chf) positive have endogenous viral genes causing expression of envelope glycoproteins of subgroup E specificity in the avian leukosis/sarcoma group. Sera from chf (+) chickens bearing subgroup A sarcomas had only marginal levels of group-specific reactivity to viral envelope glycoproteins in comparison to sera from chf (-) tumor bearing birds. The subgroup-specific reactivity in both groups was strong. The group specific determinants of the endogenous virus appeared

to induce a tolerant state in chf (+) chickens or, alternatively, resulted in binding of group specific reactive antibodies in the blood. In support of Halpern and Friis' findings, Crittenden (personal communication) has indicated that chickens without detectable ev loci are characterized by higher than average antibody responses following challenge with exogenous avian leukosis virus.

RPRL inbred lines 6-1 and 6-3 are homozygous for ev-3, a locus coding for a defective endogenous virus expressing envelope glycoproteins (Astrin et al. 1979). Both lines are characterized by a high incidence of regression of BH-RSV(RAV-1)-induced sarcomas (Collins et al. 1980). In light of the findings of Halpern and Friis, there are at least two plausible explanations for the observed high responses. The cell-mediated portion of the immune system might not have become tolerized to group specific determinants of the envelope glycoproteins. Alternatively, the B2 haplotype of these lines may be responding to envelope determinants lacking on the endogenous products or possibly to a group specific non-virion antigen (Wainberg et al. 1979a) associated with cells infected with nondefective virus.

RPRL inbred lines 7-2 and 100 contain ev-2, an endogenous virus locus which codes for the nondefective subgroup E virus, RAV-0 (Astrin et al. 1980). These chickens conceivably could be tolerant not only to chf, but also to a non-virion transformation-independent antigen

associated with virus-infected cells. Wainberg et al. (1979a) suggested that such an antigen was the major stimulus in a peripheral lymphocyte stimulation assay using lymphocytes from chickens bearing RSV-induced tumors. Results of Hall et al. (1979), hovever, implicated virus envelope antigens as the major target of immune reactivity during the primary response to RSV-induced tumors. (6-3 X 7-2)F1 chickens, which also contain ev-2, are characterized by a fairly high incidence of regression of RSV-induced tumors (Marks et al. 1979). Therefore, endogenous viruses probably have little effect if any on anti-Rous sarcoma responses of chickens carrying the B2 haplotype. It is conceivable, however, that other B haplotypes with different specificities may be influenced by endogenous ALV's.

Environmental Factors Influencing Rous Sarcoma Regression

Age

A number of environmental variables have been shown to have strong influences on Rous sarcoma regression. With increase in age of host at time of infection, there is a higher frequency of regression and a lower frequency of metastasis (Freire et al. 1953, Cotter et al. 1973, Heinzelmann et al., 1981a). In young chickens, a functionally immature immune system could clearly result in progressive tumor growth. Differences observed between

adult and six week old chickens in anti-sarcoma response, however, may reflect previous exposures to the closely related avian leukosis virus (Meyers et al. 1972) or another cross-reacting antigen such as reticuloendotheliosis virus (Baxter-Gabbard et al. 1980).

Diet

Diet has also been shown to influence tumor development. Little et al. (1948) demonstrated that development of Rous sarcoma could be completely prevented by maintaining chickens on a synthetic, folic acid free diet from the time of inoculation with virus. Treatment with folic acid antagonists produced similar results. The phenomenon was most apparent with very young chicks suggesting a direct nutritional effect on the tumor cells rather than a stimulus to the immune system. Clark (1980) demonstrated that protein-calorie restriction retarded growth of RSV-induced tumors. Restriction in tumor development and growth was thought to result from a limited supply of nutrients to the cancer cells.

Stress

There is accumulating evidence that stress, either psychological or physical, may significantly affect an individual's immune system. Mice, for example, under stressful conditions, survived for shorter periods of time than non-stressed controls when challenged with a leukemia

virus (Levine and Cohen 1959). Rats experiencing inescapable shock had a lower incidence of tumor rejection than controls following challenge with a sarcoma tumor preparation (Visintainer et al. 1982). Available evidence suggests that stress-induced immunosuppression is mediated through the action of cortisone upon lymphocytes (Monjan and Collector 1977, Eskola et al. 1978). Corticosteroids administered in vitro (Fauci and Dale 1974) suppress the response of lymphocytes to stimulation by mitogens and antigens. In addition, corticosteroids induce a decrease in the absolute numbers of circulating B and T lymphocytes, but the T-cell lymphopenia is more pronounced. It is likely that such is due to a redistribution of cells out of the circulation into other body components (Fauci and Dale 1974).

Virus dosage and type

Virus dosage can clearly influence host response to RSV-induced tumors. High dilutions of "live" RSV have been successfully used for immunization against subsequent challenge with higher concentrations of the same virus (Sigel et al. 1971). Viruses of different subgroups (McBride et al. 1981) and even different isolates of the same strain of virus (Cutting et al. 1981) demonstrate different potentials for progressive growth within a particular inbred line of chickens.

Avian leukosis virus

Rubin (1962) reported that chickens congenitally infected with avian leukosis virus required much higher concentrations of RSV to reach a tumor inducing end point than non-infected controls. When concentrations of RSV used to inoculate controls and ALV infected chickens were adjusted so that comparisons could be made on the basis of the biologically effective dosage of RSV administered, ALV congenitally infected birds invariably developed progressive tumor growth whereas tumors of controls tended to regress. Meyers and Qualtiere (1977) challenged normal chickens and chickens congenitally infected with a subgroup A ALV with strains of RSV from two different antigenic subgroups (B and C). Congenital ALV infection had no influence either on incidence or latency period of RSV-induced sarcomas, since the challenge viruses were not of the same subgroup as the ALV used for inducing congenital infection. Most tumors in RSV-challenged normal birds regressed, whereas tumors in ALV-infected birds grew progressively.

Use of ALV for immunization rather than tolerization resulted in resistance against RSV-induced tumors of the same or of different subgroups (Meyers *et al.* 1972). The protective effect was not dependent on neutralizing antibody, since chickens resistant to challenge had antibodies against only the immunizing virus and not against the heterologous challenge virus. Thus, depending on time of exposure, during embryological development or during the

immunocompetent stage of postnatal life, avian leukosis virus can either inhibit or enhance response against the closely related Rous sarcoma viruses.

Immunosuppressive infectious diseases

At the University of New Hampshire, under conditions in which the effects of host age, diet, housing conditions, and dosage and type of virus are kept uniform, there still exists considerable variability in anti-Rous sarcoma response, both within inbred lines of chickens (Collins et al. 1980) and within noninbred chickens of the same B genotype (Collins et al. 1977, Brown et al. 1982). Such variability could result from the influence of non-MHC genes or from an uncontrolled environmental variable/or variables. Since inoculated chickens are usually housed about eight to a wall cage or fifteen to a deck of a holding battery, variation in stress could result following establishment of a dominance hierarchy (Chase 1982). Variations in maternal immunity, genetic susceptibility, or in degree of exposure, would make immunosuppressive infectious diseases plausible sources of variability.

Frankel et al. (1974) reported that LSI-SPF chickens, although susceptible to intraperitoneal inoculation with Marek's disease virus, were resistant to contact exposure to MD unless concurrently exposed to ALV. Witter et al. (1975) subsequently demonstrated that dual infection with ALV, either exogenous or endogenous, was not necessary for

tumorigenesis in chickens exposed by contact to MD virus. Since both ALV (Sharma 1979) and MD (Purchase et al. 1968, Evans et al. 1971) are immunosuppressive, coincident exposure to both viruses could have conceivably resulted in a synergistic immunosuppressive effect allowing tumorigenesis to occur in the LSI-SPF chickens.

MD causes a necrotizing infection which may severely affect both the bursa and thymus (Jakowski et al. 1970) and subsequent ability to perform bursa and thymus dependent functions. Biggs et al. (1968) found that infection with MD virus increased the susceptibility of Rhode Island Red chickens to coccidial infection. Calnek et al. (1975) subsequently demonstrated that MD exposure and development of gross lesions of MD resulted in an increased incidence of tumor progression following challenge with RSV.

Infectious bursal disease (IBD) is a viral disease of poultry with a global distribution. The virus causes destruction of lymphocytes, particularly in the bursa of Fabricius but also in the thymus, spleen, and caecal tonsils (Baxendale 1981). Immunosuppression resulting from subclinical IBD infection can result in both increased susceptibility to other diseases (Rosenberger and Gelb 1978, Wyeth 1975), as well as poor responses to vaccines (Giambrone et al. 1976).

Reticuloendothelosis virus (REV) is a C-type retrovirus unrelated to viruses of the avian leukosis-sarcoma group (Purchase et al. 1973). Originally isolated from a turkey

(Theilen et al. 1966), it has subsequently been found as a contaminant of HVT (MD) vaccines (Koyama et al. 1976). REV infection results in a runting syndrome with severe atrophy of the bursa and thymus (Mussman and Twiehaus 1971). Bulow (1977) demonstrated that REV contamination markedly reduced the efficacy of MD vaccines, and Witter et al. (1979) found that the immunosuppressive effects of REV varied with the strain of virus and antigen studied.

Under conditions in which immunosuppressive infectious agents such as MD, ALV, and IBD are present, the potential of a sizable environmental influence on a chicken's anti-tumor response, or on responses to other insults such as synthetic polypeptides and bacterial antigens, is clearly evident. The possible involvement of uncontrolled environmental factors should be considered in interpretting what appear to be either MHC or non-MHC genetic influences on immune responses.

III. MATERIALS AND METHODS

Animals

Information concerning B and E haplotypes, cellular susceptibility to avian leukosis viruses, susceptibility to Marek's disease and degree of inbreeding for each of the eight different genetic stocks of chickens used in this research is given in Table 1 (pg. 28). Lines 6 subline 1 (6-1), 6 subline 3 (6-3), 7 subline 2 (7-2), 100, 15 subline 1 (15-1), and 15I subline 5 (15I-5) are inbred White Leghorn lines developed and maintained at the Regional Poultry Research Laboratory (RPRL), East Lansing, Michigan. Line UNH 105 is a noninbred line of New Hampshires originally obtained from a commercial breeder and maintained at the University of New Hampshire. Line K28, a noninbred White Leghorn line bred for susceptibility to subgroup E viruses, was provided by Harriet Robinson of the Worcester Foundation for Experimental Biology (WFEB), Shrewsbury, Mass.

Various crosses of the above lines resulted in segregation for alleles at several different blood group alloantigen loci. Typing to identify particular B, and in some cases E alloantigen genotypes was performed in the laboratory of W.E. Briles, Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois. Chickens were blood typed after two weeks of age (usually at

Table 1.[a] Characteristics of chicken lines used for generation of data

Line	B haplotype	E haplotype	Cellular susceptibility to ALV by subgroup: A	B	C	Susceptibility to Marek's disease	Inbreeding[e] coefficient
6-1	2	7	S[d]	S	S	R	.99
6-3	2	7	S	S	S	R	.99
7-2	2	5	R	R	S	S	.99
15-1	5	7	seg	S	seg	S	.99
15I-5	15	5	S	S	R	S	.99
100	2	5	seg	seg	S	S	.78
UNH 105[b]	22,23,24,26	?	S	R	seg	?	noninbred
K28[c]	13	?	S	seg	S	?	noninbred

[a]Majority of information compiled by Altman and Katz (1979).

[b]Cotter *et al.* 1973, Collins *et al.* 1979, Brown *et al.* 1982.

[c]Robinson *et al.* 1980; Robinson, personal communication.

[d]S, R, and seg represent susceptible, resistant, and segregating for susceptibility, respectively.

[e]Stone 1975.

four weeks) by using a panel of alloantisera and classifying according to the patterns of reactivity obtained (Briles *et al.* 1950).

Care of Animals

At hatching, chicks were wing-banded in the right wingweb, dubbed, and vaccinated subcutaneously with 0.2 ml of Marek's disease vaccine (cell free live turkey herpesvirus of chicken tissue culture origin, Sterwin Laboratories Inc., Millsboro, Delaware). During times of increased mortality due to a recurrent respiratory problem at the UNH Poultry Research Farm, chicks received 0.2 mg of gentamicin sulfate mixed with the Marek's vaccine. At 10 days of age, chickens received Newcastle-bronchitis vaccine (live virus of chicken embryo origin; B1 type, B1 strain - Mass. and Conn. types; Sterwin Laboratories Inc.) in the drinking water. At three weeks of age, infectious bursal disease (IBD) vaccine (live virus, chicken tissue culture origin, Agri-Bio Corp., Gainesville, Ga.) was administered in the drinking water. UNH 105 line chickens used to characterize six *B* genotypes for anti-Rous sarcoma response did not receive the IBD vaccine.

Chickens were brooded from hatching to six weeks of age in conventional, electrically-heated brooding batteries in windowless rooms at the UNH Poultry Research Farm. At six weeks of age, experimental animals were moved to

semi-isolated facilities for virus inoculation where they were maintained in holding batteries until termination of the experiment.

Breeder replacements, in addition to the above vaccination schedule, received Newcastle-bronchitis vaccine (live virus of chicken embryo origin; B1 type, La Sota strain - Mass. type; Sterwin Laboratories Inc.) in the drinking water at six weeks and again at 14 to 17 weeks of age. Breeders also received, via drinking water, Mycoplasma gallisepticum (live bacterium, F strain, grown in broth culture at UNH) and vaccine for avian encephalomyelitis (live virus, chicken embryo origin, Sterwin Laboratories Inc.) at 14 to 17 weeks of age.

Experimental chickens were fed, ad libitum, commercially prepared chick starter feed medicated with 0.004% amprolium and bacitracin methylene disalicylate. Breeder replacements were fed the starter feed until 17 to 20 weeks of age when they were transferred to special mating pens or breeder cages. At this time they were placed on a layer feed.

Viruses

The various RNA tumor viruses used and some of their characteristics are listed in Table 2. Avian leukosis virus, RAV-1 (subgroup A) and Bryan High-titer Rous sarcoma viruses of subgroups A, B, and C [(BH-RSV(RAV-1),

Table 2. Characteristics of viruses used in the various experiments.

Virus	Abbreviation	Subgroup[a]	Pathology[a]	Transmission[a]
BH-RSV (RAV-1)	RSV-1	A	sarcoma	exogenous
BH-RSV (RAV-2)	RSV-2	B	sarcoma	exogenous
BH-RSV (RAV-49)	RSV-49	C	sarcoma	exogenous
RAV-1	RAV-1	A	leukosis	exogenous
RAV-0	RAV-0	E	"nononcogenic"[b]	endogenous

[a]Tooze 1973.

[b]Motta et al. 1975, Purchase et al. 1977, Crittenden et al. 1979.

BH-RSV(RAV-2), and BH-RSV(RAV-49), respectively)] were supplied by L.B. Crittenden (RPRL) BH-RSV is a defective virus requiring the aid of an avian leukosis virus (ALV) for production of an envelope. Subgroup specificity is determined by the "helper" ALV referred to as Rous associated virus (RAV). An endogenous ALV, RAV-0, was supplied by Harriet Robinson (WFEB). It was produced by line 100 embryo fibroblasts and grown on ev-1 chicken cells susceptible to subgroup E viruses. The titer of the stock was approximately 10 million infectious units/ml. All viruses were stored in liquid nitrogen except for RAV-0 which was stored in a REVCO freezer at -70 degrees C.

Titration of Virus

The dose of virus given depended upon the experiment but was based upon an average titer for each stock virus. Two different stocks of RSV-1 were used. The first stock, used in characterizing six B genotypes of line UNH 105 for response to RSV-1 induced tumors and for testing tumor induction susceptibility of (6-1 X 15-1)F6 chickens, had a titer of approximately 200,000 pock forming units (PFU) per ml. This was based upon inoculation of the chorioallantoic membrane (CAM) of susceptible 9 to 11 day old SPAFAS chicken embryos and counting the number of foci of proliferating cells 7 days later (Hitchner et al. 1975). The diluent for this stock, both for titration and for inoculation of

experimental chickens, was Hanks' balanced salts solution containing 5% fetal calf serum plus 100 units penicillin, 100 ug streptomycin and 10 ug hyaluronidase (Sigma Chemical) per ml. The second stock of RSV-1 had a titer of approximately 1,200,000 PFU per ml. Titers of the RSV-2 and RSV-49 stocks were approximately 750,000 and 75,000 PFU per ml, respectively. Diluent for the second stock of RSV-1 and for the stocks of RSV-2 and RSV-49 was Dulbecco's Modified Eagle Medium (Gibco) with D-glucose, L-glutamine, and sodium pyruvate plus 10% tryptose phosphate broth, 4% calf serum, and 1% antibiotic-antimycotic (Gibco) resulting in 100 units penicillin, 100 ug streptomycin, and 0.25 ug Fungizone per ml. Change to this diluent was made in efforts to use the more accurate focus-forming assay (Hunter 1979) for titration of virus. The same medium was used for growth of primary and secondary cultures of SPAFAS chick embryo fibroblasts (CEF) except for the addition of 1% heat-inactivated chicken serum (Gibco).

Titers obtained using the focus assay were generally higher than those based on inoculation of CAM's. Since CAM results were available for all virus stocks used, and because a greater number of titrations were performed using CAM's, all titers above are reported in PFU's rather than FFU's (focus-forming units).

Normally stock virus was diluted one day prior to use for chicken inoculation and stored in a REVCO freezer at -70 degrees C for 24 hours. It was transported to the UNH

Poultry Farm in a frozen state, then thawed and kept on ice until time of inoculation of chickens. Titered viruses were subjected to one freeze/thaw cycle to simulate conditions of virus used for inoculation of experimental animals.

Screening the Chicken Lines for ALV

Egg albumins from (6-1 X 15-1)F4 dams were screened for presence of ALV using the complement fixation test for avian leukosis (Sarma *et al*. 1964) in the laboratory of Harriet Robinson (WFEB). Egg albumins from other lines and crosses were screened with an enzyme-linked immunosorbent assay (Smith *et al*. 1979) by E.O. Mills, Hubbard Farm, Inc., Walpole, N.H.

Virus Inoculations

Chickens in a given experiment were inoculated with a particular subgroup, or subgroups of RSV at six weeks of age. The subgroup(s) of virus and the dilution used depended upon the objective of the experiment. One tenth ml was injected subcutaneously normally into the left wingweb only. Details concerning the virus subgroup(s) used, dose, and whether one or both wings were inoculated are given for the individual experiments under Results.

Evaluation of Tumor Development and Progression

In general, tumors were scored subjectively for size at 2, 3, 4, 6, 8, and 10 weeks postinoculation (PI). Tumor score was based on the following criteria (Collins et al. 1977):

Score	Criterion
0	No palpable tumor
1	Small tumor $\leq$ 0.5 cm diameter
2	Tumor > 0.5 cm and $\leq$ 1.2 cm diameter
3	Tumor > 1.2 cm and $\leq$ 1/2 wingweb area
4	Tumor > 1/2 wingweb area but < total wingweb
5	Tumor fills wingweb
6	Massive tumor extending beyond the wingweb

A tumor profile index (TPI) was assigned on the basis of the pattern of tumor growth over the ten week postinoculation period. In most experiments a five index TPI was used, based upon the following criteria (Collins et al. 1977):

TPI	Criterion
1	Complete regression by 28 days
2	Complete regression by 56 days but after 28 days
3	Complete regression after 56 days, or a decreasing slope, or complete regression by 56 days followed by recurrence

4	General upward trend, or plateau; slight regression after 56 days
5	Terminal tumor prior to 70 days

To characterize the six B genotypes of line UNH 105 for response to RSV-1 induced tumors (Brown et al. 1982), a TPI was assigned to each chicken based upon one of three criteria. The criteria were modified to account for the different distribution of responses observed with this line, there being very few complete regressors by 28 or 56 days PI.

TPI	Criterion
1	Complete, or nearly complete, regression (tumor not exceeding 1.2 cm diameter at 70 days PI)
2	Intermediate response (tumor exceeding 1.2 cm diameter but not completely filling the wingweb at 70 days PI)
3	Terminal tumor by 70 days, or massive tumor completely filling or extending beyond the wingweb at 70 days PI)

Necropsy

Chickens dying during the experimental period were necropsied, and sections of suspect metastatic lesions were collected and processed for histological examination under the supervision of W.R. Dunlop, poultry pathologist at the University of New Hampshire.

Statistical Analyses

Mean tumor scores and TPI's with corresponding standard errors were obtained using SPSS subprograms CROSSTABS and ONEWAY (Nie *et al*. 1975). Mean TPI's adjusted for the effects of other independent variables were obtained using the "least-squares and maximum likelihood general purpose program" (Harvey 1968). Analysis of variance and *a posteriori* contrasts of means were performed using SPSS subprograms ANOVA and the modified least significant difference (LSDMOD) of subprogram ONEWAY, respectively. LSDMOD was used since it is exact for groups of unequal sizes. Chi square analyses were performed using SPSS subprogram CROSSTABS. Statistical significance was determined at $P \leq 0.05$.

In computing mean tumor scores by weeks postinoculation, any chickens dying of tumor were assigned tumor scores of 5 for the remainder of the experimental period. If cause of death was suspect, tumor being size 3 or less at time of death, no tumor scores were assigned for subsequent weeks PI. No TPI's were assigned to chickens receiving RSV inoculations in both wings or to chickens in which cause of death was suspect.

IV. RESULTS

Evidence for a Non-MHC Influence on Host Response to RSV-Induced Tumors

Genetic Influence

RPRL inbred lines 6-1, 6-3, 100, and 7-2 are all homozygous for the B2 haplotype, yet differ greatly in response to RSV-induced sarcomas. Lines 6-1 and 6-3 are regressor lines, whereas lines 100 and 7-2 have been associated with a high incidence of tumor progression (Collins et al. 1980). The objective of the following studies was to determine whether such variation was due to a non-MHC influence (genetic or environmental) or to a hitherto undetected difference at the MHC. ALV-negative dams, as determined by ELISA testing of egg albumins, were used.

To compare the B2 haplotypes of lines 7-2 and 6-1, it was necessary to cross these lines with a third line and eventually to produce a three way cross which would allow comparisons of the two B2 haplotypes on a common genetic background. Line 15I-5 was chosen for the cross for several reasons. Bacon et al. (1979) had reported that (15I-5 X 7-2)F1's were highly susceptible to avian leukosis virus. Since tumors induced by ALV would share many of the same antigens as sarcomas and since both 15I-5 and 7-2 had been

reported to be progressor lines (Collins et al. 1980), it was suspected that (15I-5 X 7-2)F1's would be characterized by progressive Rous sarcoma growth. On the other hand, a high incidence of regression was expected among (15I-5 X 6-1)F1's due to the dominant high responding B2 haplotype of line 6-1 (Collins et al. 1977). Since 15I-5 is susceptible to subgroup A sarcoma and leukosis viruses, and susceptibility is dominant, comparisons between crosses could be made using RSV-1 in spite of the subgroup resistance of line 7-2.

(15I-5 X 6-1)F1 and (15I-5 X 7-2)F1 chickens were produced by inseminating 6-1 and 7-2 dams with pooled semen from 15I-5 males. Since ALV-negative 15I-5 dams were not available, the reciprocal matings of 6-1 and 7-2 males with 15I-5 females were not made. Progeny were challenged in the left wingweb with approximately 1200 PFU of RSV-1 and in the right wingweb with RSV-49 (approximately 75 PFU) at six weeks of age. The two sets of F1 generation progeny differed greatly in anti-tumor response over the 10 week period postinoculation (Figure 1A, page 45). RSV-1 induced sarcomas were the same size at two weeks PI for both groups, but thereafter (15I-5 X 6-1)F1's rapidly regressed their tumors, whereas (15I-5 X 7-2)F1's developed progressive tumor growth. Chickens dying as a result of RSV-1 induced tumor burden were scored as size 5 throughout the remainder of the postinoculation period. If cause of death was suspect, no tumor score was assigned to that chicken in

computation of mean tumor scores in succeeding weeks. Of 31 (15I-5 X 6-1)F1's inoculated with RSV-1, all developed tumors, one died of suspect causes, and all the rest (97%) rapidly regressed their tumors. Of 32 (15I-5 X 7-2)F1's, all developed RSV-1 induced tumors, three died of suspect causes, and 26 (81%) died with progressive tumor growth. Suspect causes of death were those in which tumor size at time of death was small (size 3 or less).

Response to the RSV-49 induced tumors is depicted in Figure 1B (page 45). Mean tumor scores for (15I-5 X 7-2)F1's are reported only through the fourth week PI due to high mortality resulting from massive sarcomas induced by RSV-1. Using RSV-49, (15I-5 X 6-1)F1's had 100% tumor induction with size 3 or 4 tumors at two weeks PI and subsequent rapid tumor regression. In contrast, (15I-5 X 7-2)F1's demonstrated resistance to tumor induction with rs.V-49, only 5 of 32 developing tumors. (Chickens which did not develop right-wing tumors were not included in computation of mean tumor scores depicted in Figure 1B.) Tumors which did develop were small, size 1 or 0, at two weeks PI. Resistance to tumor induction with RSV-49 had been observed with an earlier hatch of the same genetic stocks (No comparative regression data was obtained from this hatch because of this resistance.). Of eighteen 7-2 and ten (15I-5 X 7-2)F1 chickens inoculated at 6 weeks of age with approximately 75 PFU of RSV-49, only four 7-2 and no (15I-5 X 7-2)F1 chickens developed tumors by two weeks

PI. (All four tumors were size one.) In contrast, all nine (15I-5 X 6-1)F1 progeny inoculated with the same virus developed tumors, only one of which was less than size three at two weeks PI. Of twelve 7-2 and eight (15I-5 X 7-2)F1 "no-takes" rechallenged with a 10-fold greater dosage of RSV-49, 17 developed tumors.

The above study confirmed an obvious difference between 7-2 and 6-1 in their associated anti-tumor responses, but did not localize the source of variation. To better evaluate the B2 haplotype of 7-2, ELISA negative 7-2 dams were inseminated with (15I-5 X 6-3)F1 semen. The resulting (15I-5 X 6-3)F1 X 7-2 progeny were blood typed to identify the segregating B2/B2 and B2/B15 genotypes. They were then challenged as above in both left and right wings with approximately 1200 PFU RSV-1 and 75 PFU RSV-49, respectively. Mean RSV-1 and RSV-49 induced tumor scores (Figures 2A and 2B, respectively; page 46) for B2/B2 and B2/B15 chickens were compared over the ten-week period PI. Both genotypes had a high incidence of regression with both subgroups of RSV. Since B15 has been associated with low response to RSV-1 (see (15I-5 X 6-3)F2 data in Figure 8A), the regression associated with the B2/B15 genotype was attributed to the B2 haplotype from 7-2 (B2 in a B2/B15 genotype could only come from 7-2.). Thus the high incidence of progression previously observed in B2/B15 (15I-5 X 7-2)F1's was the result of a non-MHC influence.

In spite of the negative ALV status of the 7-2 dams and

the generally infrequent occurrence of subgroup C ALV congenital infections (Tooze 1973), it is conceivable that an undetected low level congenital infection with subgroup C ALV could have resulted in both resistance to tumor induction with RSV-49 due to virus receptor interference, and progressive sarcoma growth due to tolerance. Among the 35 (15I-5 X 6-3)F1 X 7-2 chickens in the above experiment, 15 had size 2 or less RSV-49 induced sarcomas at 2 weeks PI. Mean RSV-1 induced tumor scores throughout the postinoculation period for these chickens were similar to those of (15I-5 X 6-3)F1 X 7-2 chickens with larger RSV-49 induced tumors the second week PI (Figure 3A, page 47). The relative susceptibility to tumor induction with subgroup C sarcoma virus appears unrelated to ability to respond to tumor induction with subgroup A RSV. The small RSV-49 induced tumors (at 2 weeks PI) rapidly regressed (Figure 3B, page 47), results inconsistent with congenital infection with subgroup C ALV.

Concurrently with the (15I-5 X 6-3)F1 X 7-2 chickens, 6-1 X (15I-5 X 6-3)F1 and (15I-5 X 6-3)F1 X 6-3 chickens were inoculated with the same two viruses at the same time. Figures 4A and B (page 48) compare responses of (15I-5 X 6-3)F1 X 7-2 chickens with those of the combined 6-1 X (15I-5 X 6-3)F1 and (15I-5 X 6-3)F1 X 6-3 chickens to tumors induced by RSV-1 and RSV-49 respectively. Response against both RSV-1 and RSV-49 induced sarcomas appears to be more rapid in the pooled crosses to 6-1 and 6-3 than in (15I-5 X

6-3)F1 X 7-2 chickens. Thus Rous sarcoma regression appears to be a quantitative rather than qualitative trait.

Further evidence for a non-MHC influence on Rous sarcoma regression was obtained using line 100 and B23/B23 line 105 chickens to produce F1 and subsequent F2 and backcross progeny. A spectrum of responses associated with different B genotypes had been previously observed in line 105 (Collins et al. 1979, Brown et al. 1982). Therefore the background associated with this line did not in general appear to be preventing expression of regressor phenotypes. If low response associated with the B2 haplotype of line 100 was the result of genetic background, then changing the background could conceivably change the phenotype associated with this B haplotype.

(100 X B23/B23 105)F2 and backcross progeny from ALV-negative dams (as determined by ELISA testing of egg albumins) were blood typed to identify B genotypes B2/B2, B2/B23, and B23/B23. Three different subgroups of RSV were used for wingweb inoculation at six weeks of age. Virus subgroups were assigned to consecutive wing band numbers of progeny of the three mating types. Each chicken was inoculated in the left wingweb with 1/10 ml of one of the following: a 1:100 dilution of RSV-1 (approximately 1200 PFU), a 1:10 dilution of RSV-2 (approximately 7500 PFU), or a 1:100 dilution of RSV-49 (approximately 75 PFU).

Among F2 chickens, the B23/B23 genotype gave the lowest anti-tumor response against the RSV-1, RSV-2, and RSV-49

induced tumors (Figures 9A, B, and C, respectively; page 68). Combining data of B2 homozygotes and heterozygotes, the influence of non-MHC genetic background can be clearly seen in Figures 5A, B, and C (page 49). Highest responses against all three subgroups of RSV are consistently associated with F1 X 105 progeny (B2/B23), whereas lowest responses are associated with F1 X 100 progeny (B2/B2 and B2/B23). Results of analysis of variance for the B2/B2 and B2/B23 genotypes in the F2's and backcrosses are provided in Table 3A (page 50). Responses were evaluated on the basis of a 5 index TPI (see Materials and Methods). Mating type can be seen to be a significant source of variation ($P \leq 0.05$). Mean TPI's for the combined B2 homozygotes and heterozygotes (Table 3B, page 50) demonstrate the mating type effect.

Environmental Influences

Avian leukosis virus

Increased resistance to tumor induction had become an increasingly greater problem among several genetic stocks maintained at UNH. Certain dams consistently produced progeny susceptible to RSV-1, whereas others produced chicks resistant to sarcoma induction. In general, tumors which did develop, appeared later in siblings of resistant chicks than in chicks from high susceptibility families (Table 4, page 52). A COFAL (COmplement Fixation test for Avian Leukosis) test of a total of twelve eggs from eleven (6-1 X

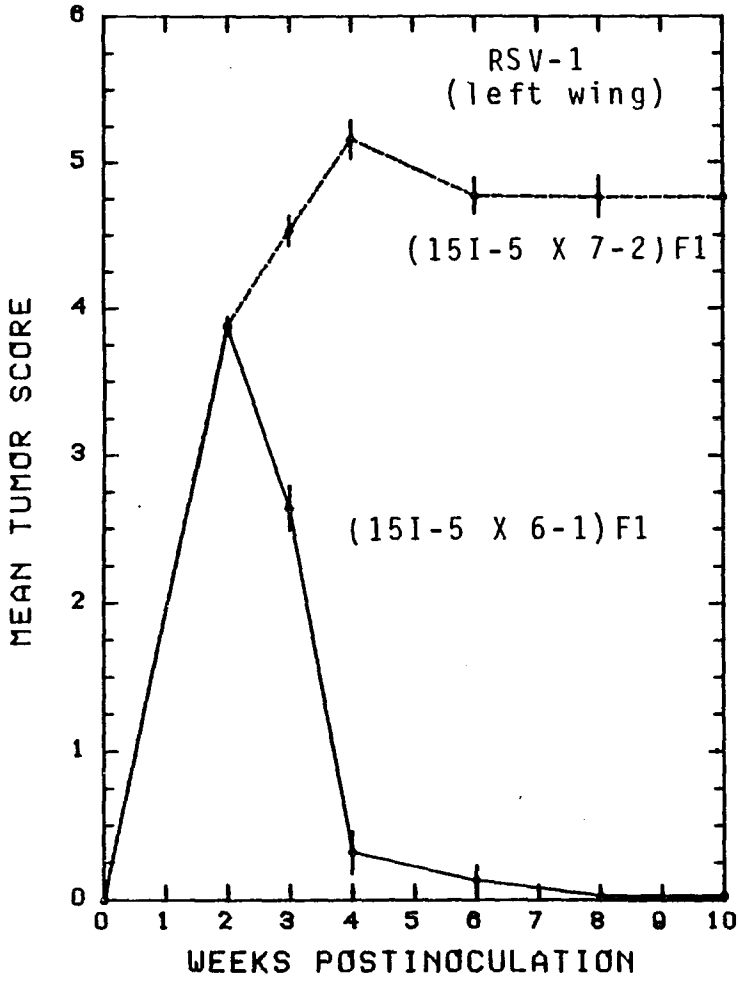

Figure 1A

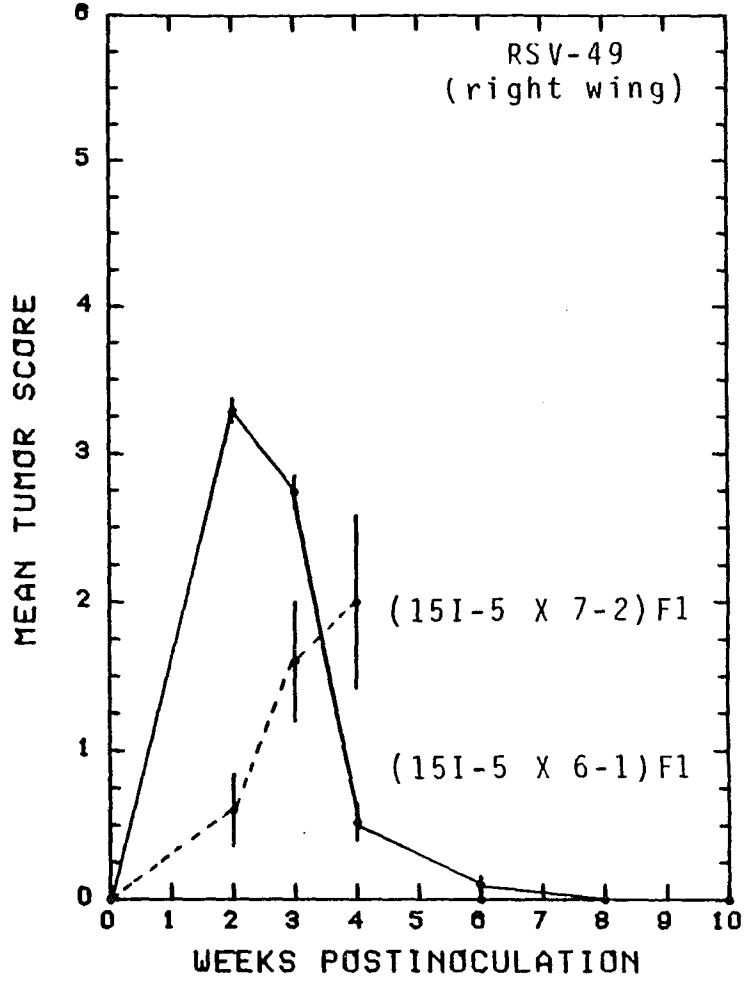

Figure 1B

Figures 1A and B.

Mean left (Fig. 1A) and right (Fig. 1B) wing tumor scores by weeks post-inoculation for (15I-5 X 6-1)F1 and (15I-5 X 7-2)F1 chickens (solid and dashed lines, respectively) challenged in the left wingweb with RSV-1 and in the right with RSV-49. Standard errors are represented by vertical lines.

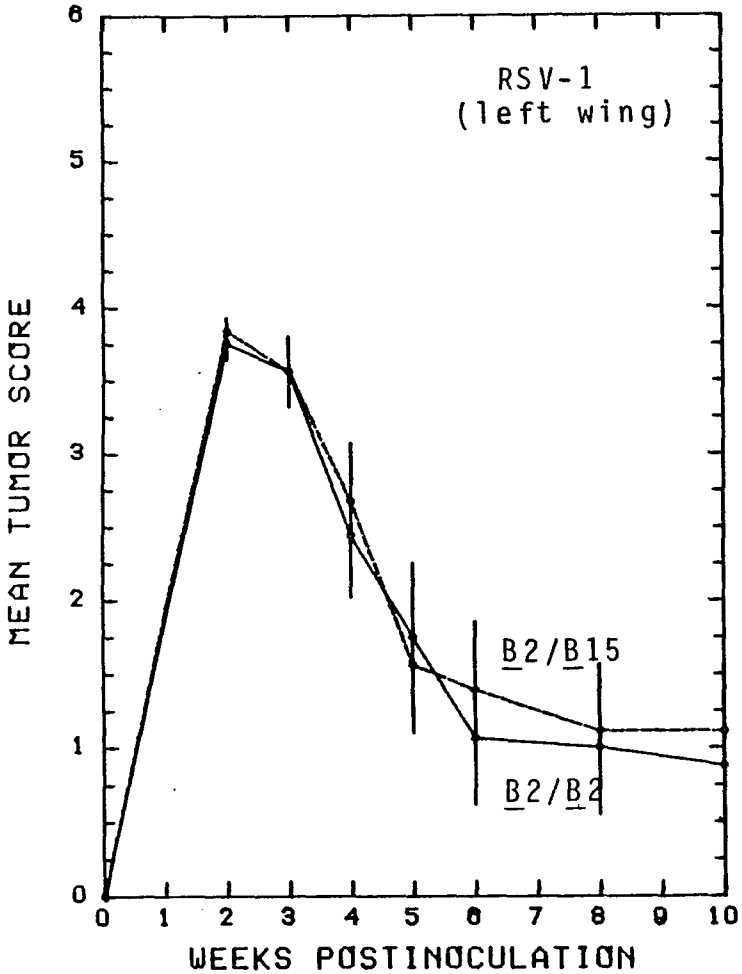

Figure 2A

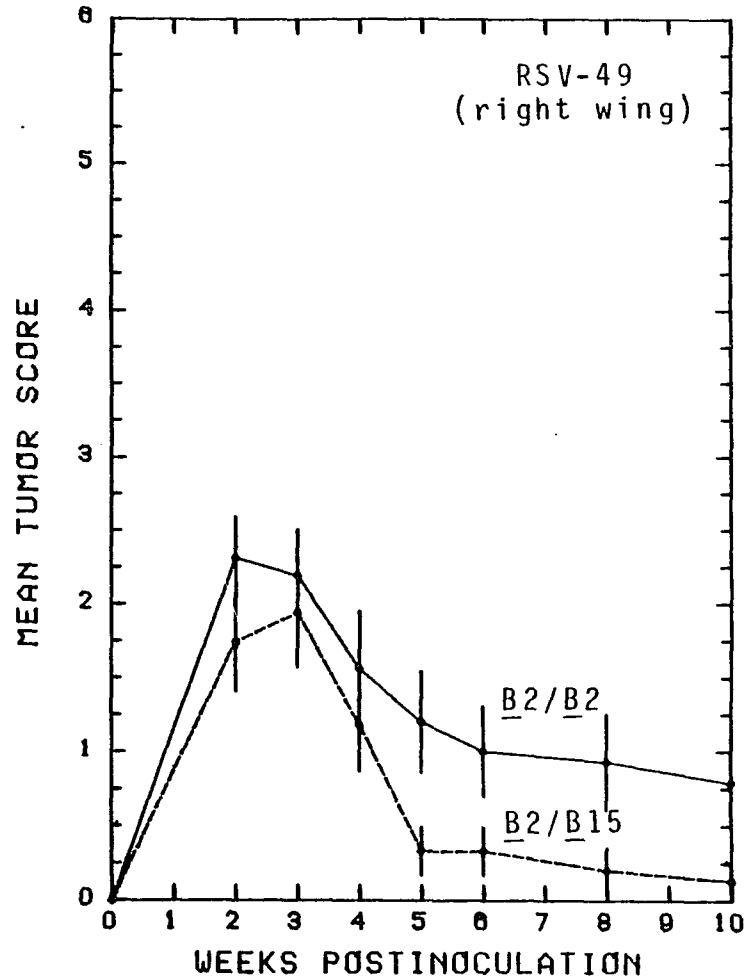

Figure 2B

Figures 2A and B.
Mean left (Fig. 2A) and right (Fig. 2B) wing tumor scores by weeks post-inoculation for B2/B15 (dashed lines) and B2/B2 (solid lines) (15I-5 X 6-3)F1 X 7-2 chickens challenged in the left wingweb with RSV-1 and in the right wingweb with RSV-49. Standard errors are represented by vertical lines.

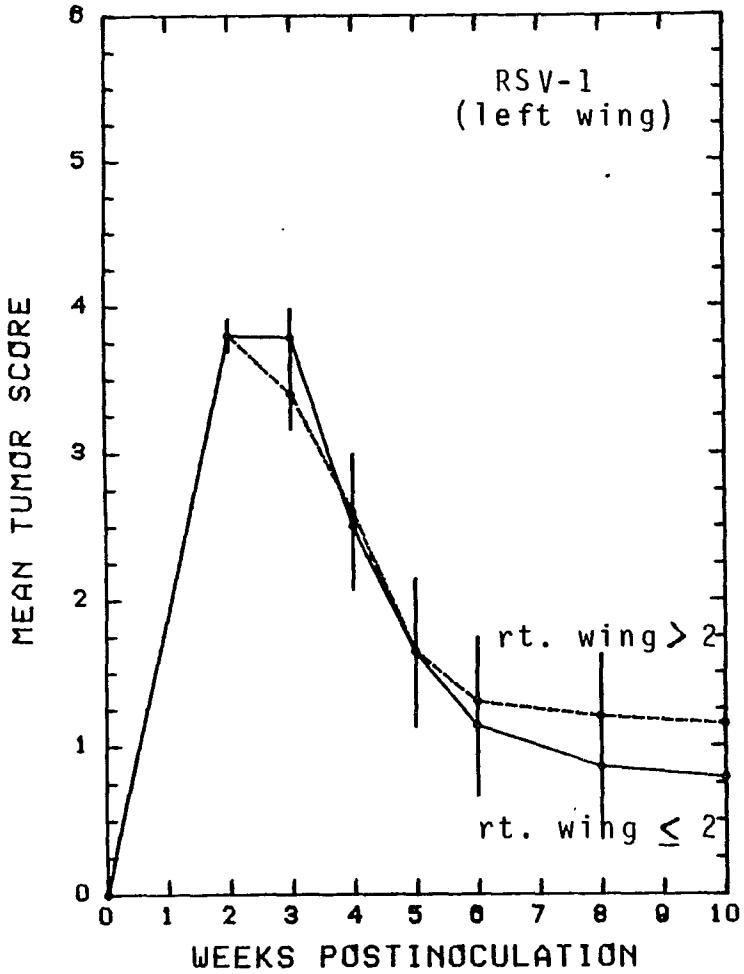

Figure 3A

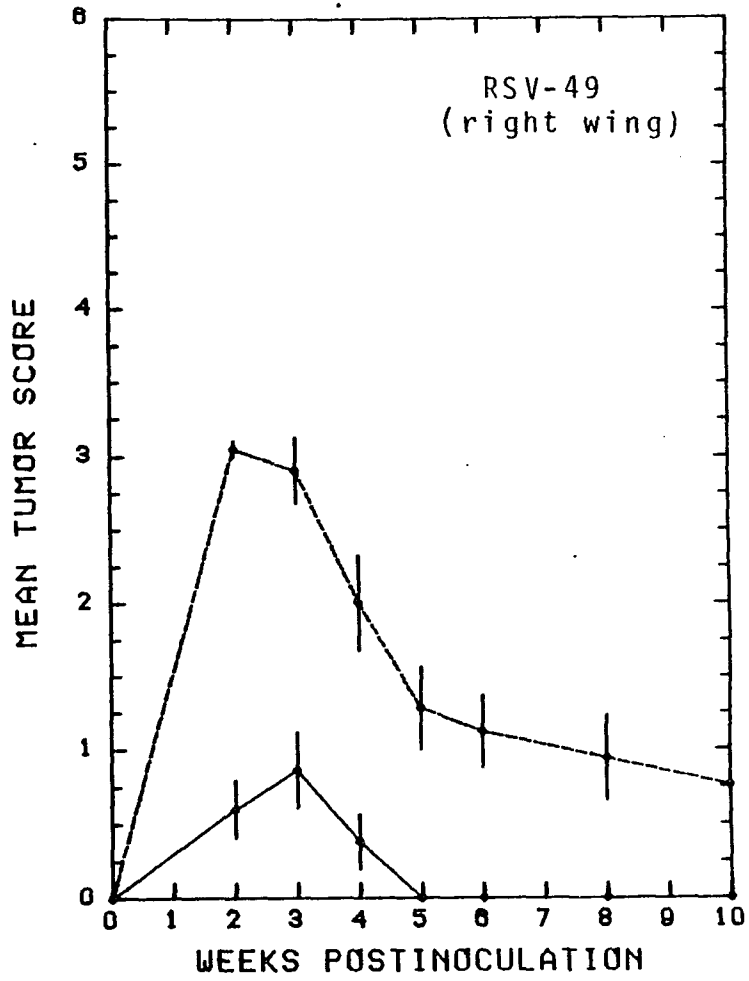

Figure 3B

Figures 3A and B.

Mean left (Fig. 3A) and right (Fig. 3B) wing tumor scores by weeks post-inoculation for (15I-5 X 6-3)F1 X 7-2 chickens challenged in the left wingweb with RSV-1 and in the right with RSV-49. Solid lines represent chickens with small (size 2 or less) RSV-49 induced tumors at 2 weeks PI. Chickens with larger RSV-49 induced tumors (greater than size 2) at 2 weeks PI are represented by the dashed lines. Standard errors are indicated by the vertical lines.

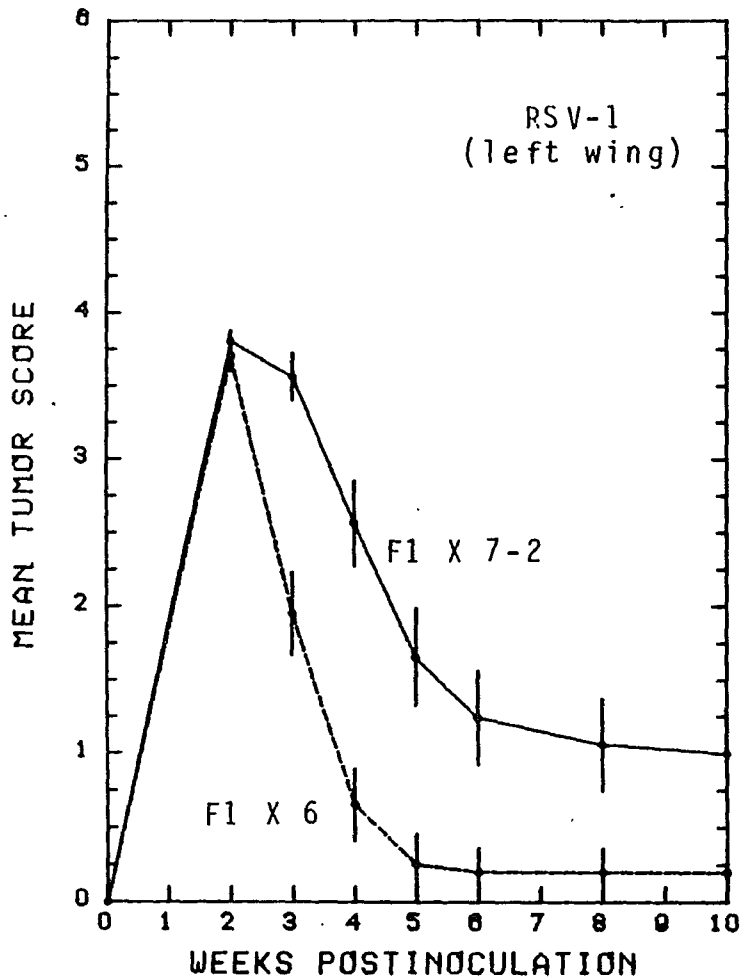

Figure 4A

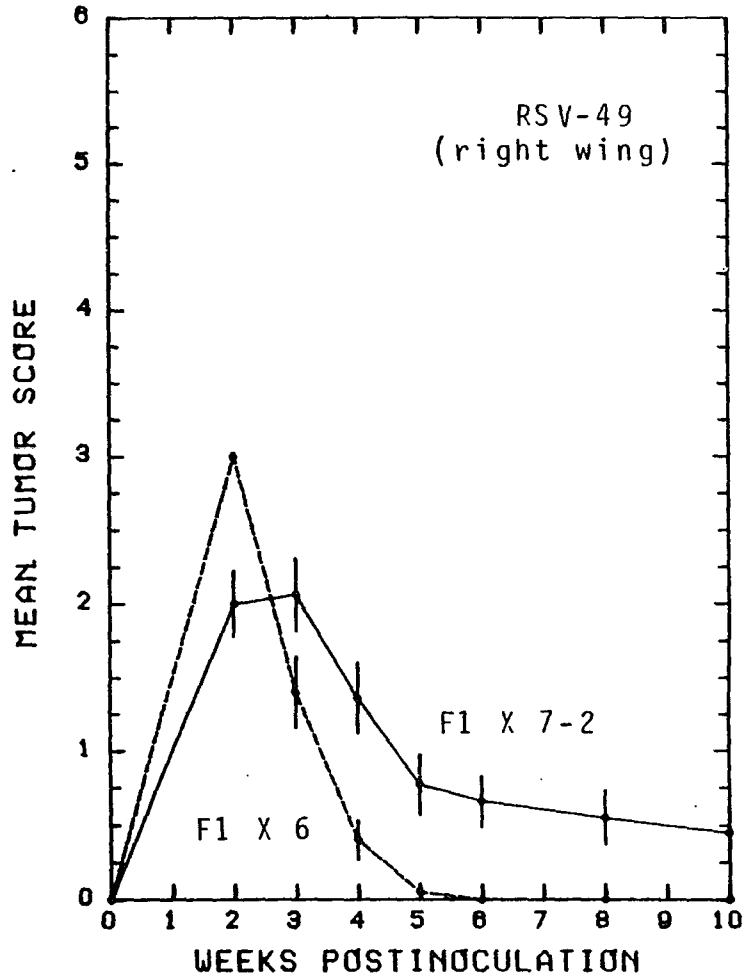

Figure 4B

Figures 4A and B.

Mean left (Fig. 4A) and right (Fig. 4B) wing tumor scores by weeks post-inoculation for [(15I-5 X 6-3)F1 X 7-2] and [(15I-5 X 6-3)F1 X 6-1 or 6-3] chickens (solid and dashed lines, respectively) challenged in the left wingweb with RSV-1 and in the right wingweb with RSV-49. Standard errors are indicated by vertical lines.

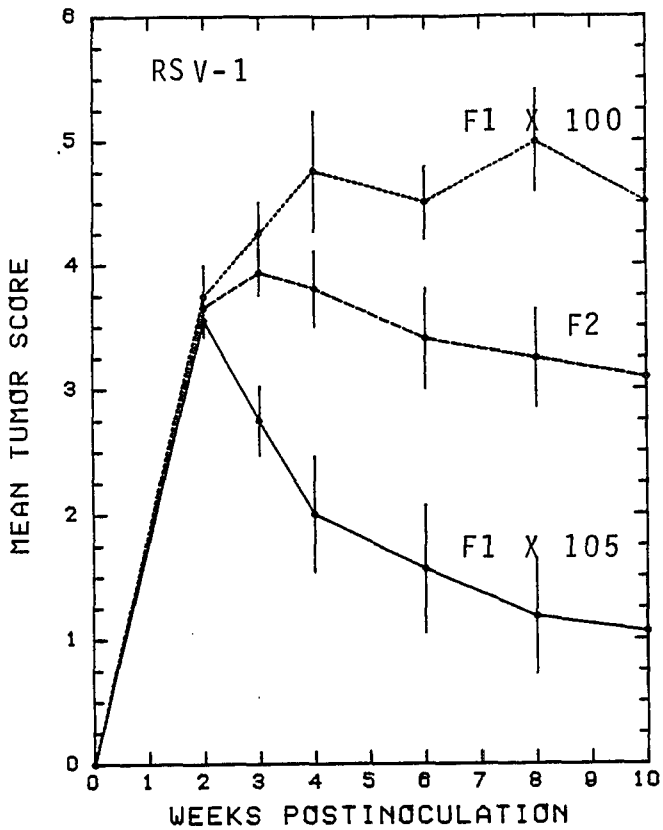

Figure 5A

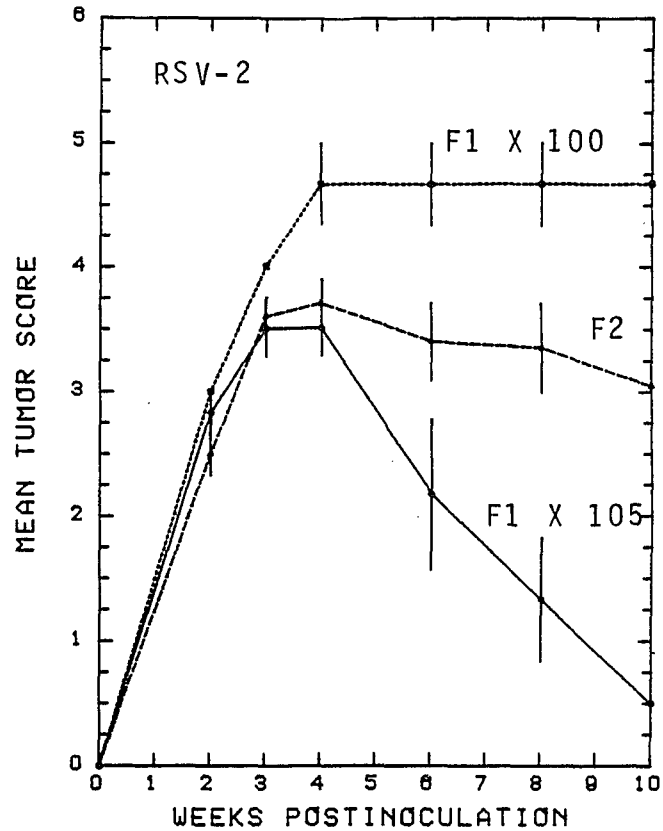

Figure 5B

Figures 5A, B, and C.

Mean tumor scores by weeks postinoculation for B2 homozygous and heterozygous chickens from (100 X B23/B23 105)F2 and backcross matings. F2, F1 X 100, and F1 X 105 chickens (dashed, dotted, and solid lines, respectively) were inoculated in the left wingweb RSV-1, RSV-2, or RSV-49, responses to which are depicted in Figures 5A, B, and C, respectively. Standard errors are indicated by the vertical lines.

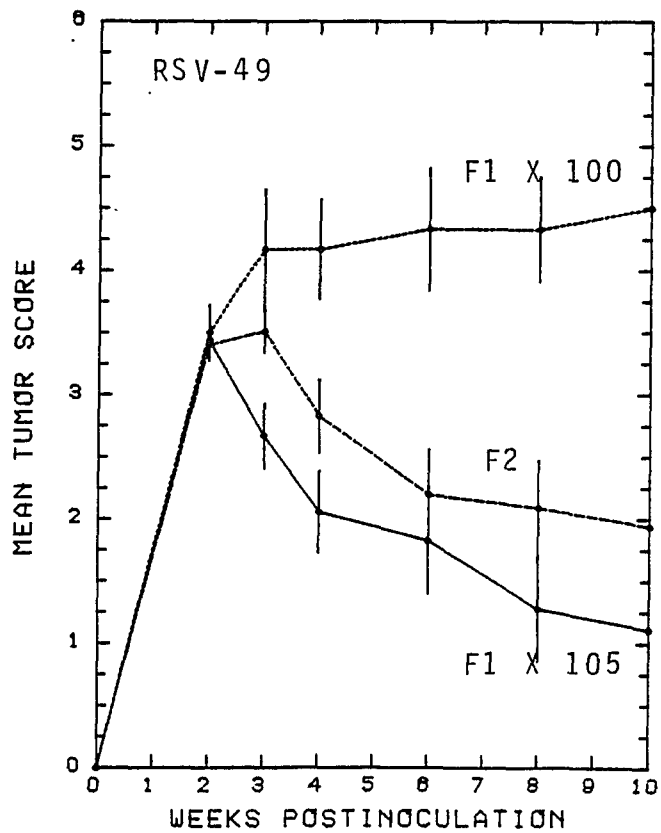

Figure 5C

Table 3A. Analysis of variance of TPI's from B2/B2 and B2/B23 chickens produced by (100 x B23/B23 105)F2 and both backcross matings.

Sources of variation	d.f.	Mean squares
Genotype	1	0.226
Sex	1	0.560
Hatch	1	4.845
Virus	2	3.230
Mating type	2	24.963*
Residual	132	1.374

*Probability of associated F $\leq$ 0.05.

Due to low egg production, the F1 x 100 backcross produced no progeny for the second hatch. Because of empty cells, interactions were not calculated in the analysis.

Table 3B. Mean TPI's by virus and mating type for B2 homozygous and heterozygous chickens from (100 x B23/B23 105)F2 and backcross matings.

Mating type	Virus	No. of chickens inoculated	No. of tumors	Mean TPI
(100 x B23/B23 105)F_2	RSV-1	32	32	3.9
	RSV-2	29	20	4.0
	RSV-49	35	35	3.2
F1 x 100	RSV-1	4	4	4.8
	RSV-2	5	3	4.7
	RSV-49	6	6	4.7
F1 x 105	RSV-1	16	16	2.2
	RSV-2	14	6	2.8
	RSV-49	18	18	2.5

15-1)F5 dams revealed only four negative eggs. All four had come from dams producing chicks susceptible to tumor induction with RSV-1. The remaining eight eggs were from dams that had been producing chicks resistant to tumor induction. Since it has been well documented that congenital infection with ALV results in resistance to tumor induction with sarcoma virus of the same subgroup (Tooze 1973), it appeared that congenital infection with a subgroup A ALV was the cause of the observed resistance to tumor induction with RSV-1 (RSV-1 being subgroup A).

The effect of ALV infection of dams on the response of their reciprocal (100 X B23/B23 105)F1 progeny to RSV inoculation, tumor size at two weeks PI and ultimately TPI, was investigated. Egg albumins from the dams were ELISA tested for the presence of ALV. Six-week-old progeny from both ALV-positive and ALV-negative dams were inoculated in the left wingweb with RSV-1 (approximately 1200 PFU). Failure to induce a tumor by two weeks PI (tumor score of zero) was markedly more frequent in progeny from ALV-positive than from ALV-negative dams (Table 5, page 53). Chickens which failed to develop tumors by the third week PI were terminated. The distribution of progeny to TPI's according to the ALV status of their dams showed that the incidence of tumor progression (TPI's of 4 and 5) was significantly higher in the progeny of ALV-positive dams.

Table 4. Percent distribution of (6-1 x 15-1)F6 chickens to tumor score according to family susceptibility to RSV-1

Status of family for susceptibility to RSV-1	Number of Progeny	Percent distribution of chickens according to tumor score (9 days PI): 0	1	2	3	4	5
Susceptible	26	--	--	--	4	96	--
Resistant	76	50	18	12	13	4	3

Chickens were inoculated in the left wingweb at 20 days of age with RSV-1 (1:10 dil. of stock). Four dams consistently producing RSV-1 susceptible progeny produced the 26 chickens in the susceptible class. Chickens in the resistant class were from 17 different dams that produced both resistant and susceptible progeny.

Table 5. Distribution of F1 progeny from reciprocal matings of line 100 by B23/B23 105 according to RSV-1 induced tumor score (2 weeks PI), TPI, and ALV status of their dams.

Tumor score (2 weeks PI)	Number of progeny: ALV positive dams	ALV negative dams	TPI	Number of progeny: ALV positive dams	ALV negative dams
0	23	1	1	1	1
1	3	0	2	16	47
2	2	1	3	11	7
3	27	20	4	10	2
4	39	43	5	29	6
	94	65		67	63

$X^2 = 26.5$; $P \leq 0.05$ $X^2 = 20.1$; $P \leq 0.05$

Marek's disease

Although both replacements and experimental chickens were vaccinated at hatching for Marek's disease (MD), occasionally chickens did succumb to the disease. Since MD virus is known to be immunosuppressive (Jakowski et al. 1970, Calnek et al. 1975), infection could result in decreased ability to respond to RSV-induced tumors.

As previously mentioned, some chickens in the experiment comparing (15I-5 X 6-1)F1 and (15I-5 X 7-2)F1 responses to RSV died of suspect causes (page 40), tumors being size three or less at time of death. Unfortunately, no sections of the bursas were obtained from these to check for MD or IBD (infectious bursal disease) involvement. However, bursal sections were obtained from four (15I-5 X 7-2)F1 chickens dying with large sarcomas. Three of the four had lesions characteristic of MD (Calnek and Witter, 1978).

In the experiment involving (15I-5 X 6-3)F1 X 7-2 chickens (page 41), it was planned that all chickens dying during the postinoculation period would have bursal sections collected for histological examination. Only six chickens died. Bursal sections were obtained from four of them. Of these four, two had lesions characteristic of MD.

Figure 6A (page 56) is a photograph of a histological section from the bursa of a ten-week-old line 105 chicken that had died of progressive Rous sarcoma growth. Aside from slight post-mortem changes, the bursa exhibits normal

architecture. Figure 6B is a photograph of a section from the bursa of a (15I-5 X 6-3)F1 X 7-2 chicken that had died with progressive sarcoma growth. The marked proliferation of interfollicular connective tissue and diffuse infiltration with lymphocytes is characteristic of MD (Calnek and Witter 1978).

A hatch effect was observed in an experiment characterizing (15I-5 X 6-3)F2 chickens from ALV-negative dams for their response to each of three subgroups of RSV. (More details of the experiment and results are provided in the following section describing the influence of virus subgroup on anti-Rous sarcoma response.) There were a total of four hatches. Tables 6A and B (page 57) were compiled from the combined data of the regressor genotypes, B2/B2 and B2/B15. The rank order of hatches for mean TPI is approximately the same for all three subgroups of RSV. No such pattern is observed with mean tumor scores at two weeks PI. Thus fluctuations in virus titer from hatch to hatch would not explain the lower responses to RSV (higher mean TPI's) observed in hatch one. Known exposure to MD virus occurred with this hatch since four (6-1 X 15-1)F2 chickens housed with them died of MD shortly before the RSV inoculation date. The (15I-5 X 7-2)F1,s previously described and this hatch of (15I-5 X 6-3)F2's had the same hatch date, were raised in the same building, were inoculated with RSV on the same day, and maintained in the same isolation room during the postinoculation period.

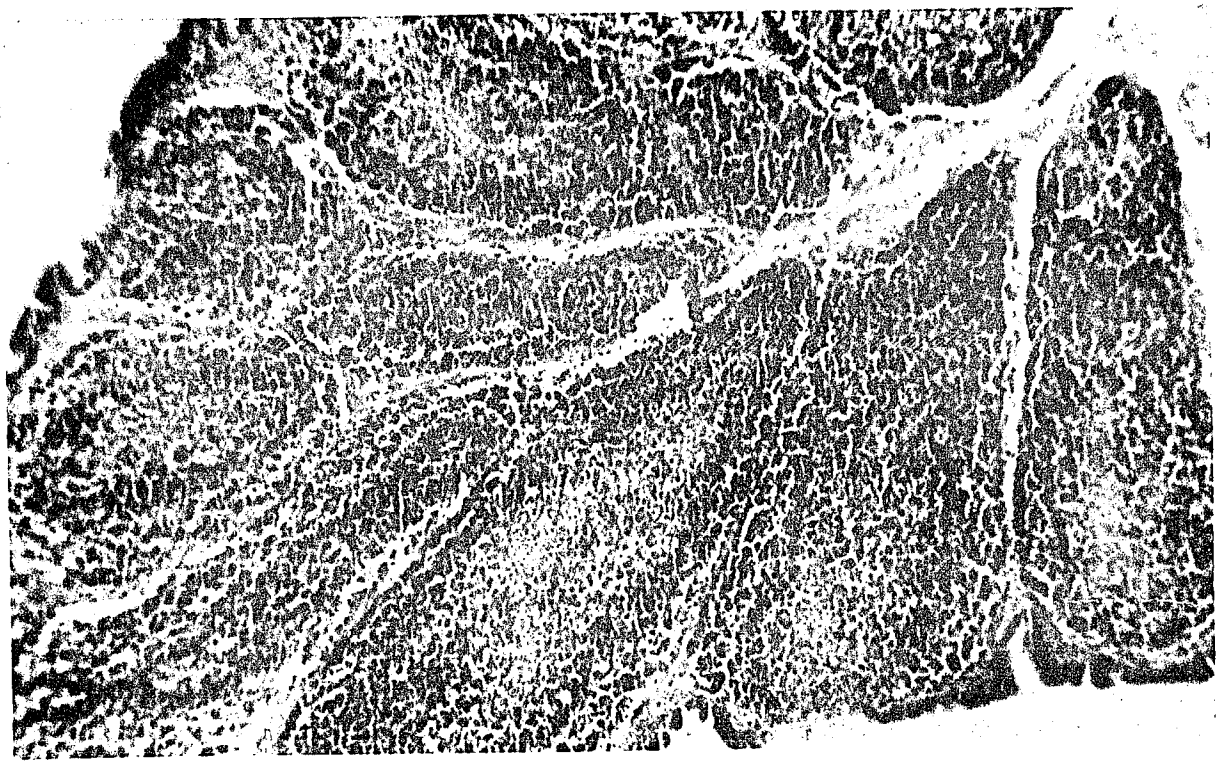

Figure 6A
Bursa from a ten-week-old line 105 chicken that had died of progressive Rous sarcoma growth. Aside from slight post-mortem changes, the bursa appears normal. H & E; X 100.

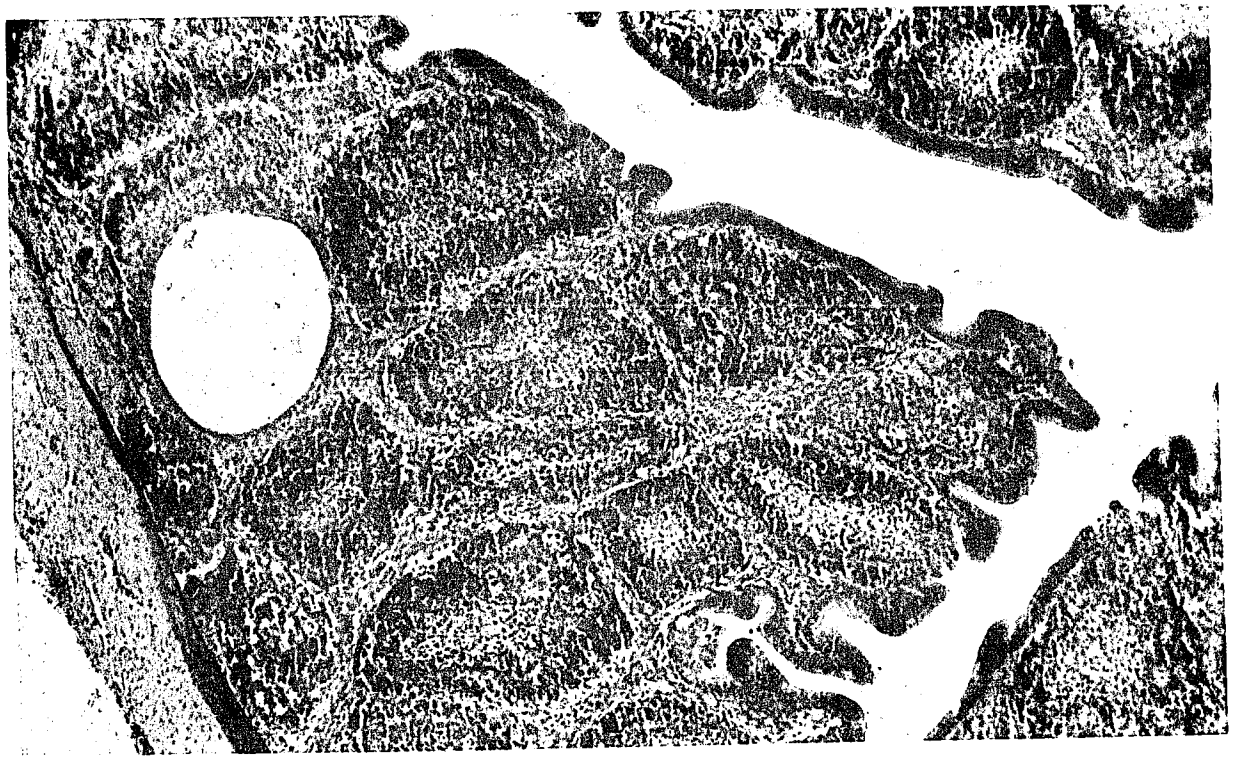

Figure 6B
Bursa from a ten-week-old (15I-5 X 6-3)F1 X 7-2 chicken that had died of progressive Rous sarcoma growth. H & E; X 100.

Table 6A. Mean TPI's and tumor scores (2 weeks PI) by virus and hatch, combined data of B2/B2 and B2/B15 chickens from a (15I-5 x 6-3)F2

Hatch	Mean tumor profile index: RSV-1	RSV-2	RSV-49	Mean tumor score at two weeks PI: RSV-1	RSV-2	RSV-49
1	2.2	3.5	2.9	3.8	2.7	3.3
2	1.7	2.6	1.8	3.5	3.0	3.3
3	2.2	3.0	1.8	3.8	3.0	3.1
4	1.6	2.6	1.6	3.6	2.7	3.3

Table 6B. Analysis of variance of TPI's from B2/B2 and B2/B15 (15I-5 x 6-3)F2 chickens challenged with RSV-1 RSV-2, or RSV-49

Sources of variation	d.f.	Mean squares
Genotype	1	0.579
Sex	1	0.327
Hatch	3	3.105*
Virus	2	16.708*
Residual	185	0.870

*Probability of associated $F \leq 0.05$

Since none of the two or three-way interactions were significant, such were not included in the table.

Influence of virus subgroup on anti-tumor response

RSV-transformed avian cells have been shown to express a variety of antigens relevant to the immune response (Ignjatovic et al. 1978, Hall et al. 1979). Therefore, response against tumor cells could involve interactions with a wide spectrum of antigenic determinants, each contributing in varying degree to the observed anti-tumor response. The specificity of B genotype response to RSV-induced tumors was investigated with four different F2 populations of chickens. Each F2 population was challenged with three different subgroups of RSV (RSV-1, RSV-2, and RSV-49 of subgroups A, B, and C respectively). The F2 populations, the B genotypes for which they were segregating, the number of hatches, ALV status of dams, and total number of chickens for each F2 population are provided in Table 7 (page 63). The F2 populations used were chosen mainly on the basis of availability and for the potential of gaining additional information such as presence or absence of MHC complementation and the effect of B genotype on frequency of metastasis. The different populations also provided a type of replication of the influence of virus subgroup which would tend to allow a broader interpretation of results.

All chickens were blood typed for identification of B genotypes and RSV inoculated in the left wingweb at six weeks of age. Each chicken received 1/10 ml of one of the three sarcoma viruses. Dosages were approximately 1200 PFU, 7500 PFU, and 75 PFU for RSV-1, RSV-2, and RSV-49

respectively. Tumors were scored as usual over the ten-week postinoculation period, and TPI's were assigned using a 5 index TPI. Analysis of variance was performed for each F2 population to determine presence or absence of a significant B genotype by virus interaction.

Results from (6-1 X 15-1)F2's. Mean tumor scores for (6-1 X 15-1)F2 chickens over the ten-week period postinoculation are shown for RSV-1, RSV-2, and RSV-49 induced tumors in Figures 7A, B, and C, respectively (page 64). B5/B5 chickens were characterized by progressive tumor growth with each of the three viruses. B2/B2 chickens rapidly regressed RSV-1 and RSV-49 induced sarcomas. Response against RSV-2 was intermediate. Response of B2/B5 heterozygotes for all three virus-induced tumors was similar to, but slightly lower than, that of the B2/B2 homozygotes. Tumor size at two weeks PI (mean score < 3) indicated that the lower response to RSV-2 induced tumors was not due to an overwhelming dose of virus. The analysis of variance, Table 8A (page 65), indicates a significant genotype by virus interaction influencing TPI. Least squares mean TPI's by B genotype and virus are given in Table 8B. Highest TPI's (lowest responses) for B2/B2 and B2/B5 chickens are associated with RSV-2.

Among the (6-1 X 15-1)F2 chickens, there was a fairly high incidence of resistance to both RSV-1 and RSV-49 induced tumors (38 and 25%, respectively). Resistance to RSV-1 was expected in light of the high incidence of ALV

contamination among the F1 dams (Table 7, page 63) and the relatively frequent occurrence of congenital transmission of ALV's of subgroup A (Tooze 1973). As expected, only a few (8%) of the chickens resistant to RSV-1 came from ALV-negative dams. In contrast, 29% of chickens resistant to RSV-49 came from ALV-free dams (the approximate percentage expected on a purely random basis).

To investigate the possibility of undetected ALV contamination with a subgroup C virus, sixty B2/B2 (6-1 X 15-1)F2 chickens from ALV negative dams (as judged by ELISA testing of albumins) were challenged at six weeks of age with approximately 1200 PFU RSV-1 in the left wingweb and 75 PFU RSV-49 in the right. All chickens developed size 3 or 4 RSV-1 induced tumors by two weeks PI. Fourteen of the sixty chickens (23%) appeared to be resistant to tumor induction with RSV-49, tumors being small (size 1) or absent at two weeks PI. Mean RSV-1 induced tumor scores for the second, third, and fourth weeks PI for these fourteen chickens were similar to those of the other 46 with larger (size 3) RSV-49 induced tumors the second week PI (Figure 10A, page 71). The small RSV-49 induced tumors rapidly regressed (Figure 10B). Thus variation in susceptibility to tumor induction with RSV-49 did not appear to be the result of congenital contamination with a subgroup C ALV.

Results from (15I-5 X 6-3)F2's. Combined data of four hatches of (15I-5 X 6-3)F2 chickens from ALV-free dams gave the mean tumor scores in Figures 8A, B, and C (page 66).

B2/B2 and B2/B15 chickens had nearly identical responses to all three viruses. They rapidly regressed both RSV-1 and RSV-49 induced sarcomas. They also regressed RSV-2 induced sarcomas, but at a slower rate. B15/B15 responses against RSV-1 and RSV-2 induced sarcomas were relatively low, whereas the corresponding response to RSV-49 induced tumors was characterized by fairly rapid tumor regression from the second to the fourth week PI with a subsequent plateau in response. The results of the analysis of variance are given in Table 9A (page 67). Again there is a significant genotype by virus interaction. The adjusted least squares mean TPI's for each B genotype against each of the three virus induced tumors (Table 9B) demonstrate the interaction effect.

Results from (100 X B23/B23 105)F2's. Mean tumor scores for (100 X B23/B23 105)F2 chickens (from ALV-negative dams) over the ten-week postinoculation period are depicted for RSV-1, RSV-2, and RSV-49 induced sarcomas in Figures 9A, B, and C, respectively (page 68). B2 homozygotes and heterozygotes had lower responses to all three virus induced tumors than associated with the (6-1 X 15-1)F2 and (15I-5 X 6-3)F2 populations described above. This depressed response was attributed to a non-MHC influence associated with line 100 (see Figures 5A, B, and C; page 49). B23/B23 response was low against all three virus induced tumors. The results of the analysis of variance are given in Table 10A (page 69). No significant genotype by virus interaction is

indicated. The distribution to TPI for (100 X 105)F2 B2/B2 homozygotes (Table 10B), however, appears to be similar to that found in the other F2 populations, lowest response being associated with RSV-2 induced sarcomas.

Results from [B5/B5 (6-1 X 15-1)F5 X B24/B24 105]F2's. B5/B5 (6-1 X 15-1)F5 chickens were crossed with B24/B24 line 105's. The resulting "F1" population was then used in a flock mating to produce two hatches of F2 progeny. Table 11 (page 70) provides the distribution to TPI, by genotype, for each tumor virus. All three B genotypes were characterized by progressive tumor growth for each of the challenge viruses. The lack of resistance to tumor induction with RSV-1 in these chickens was not expected in light of probable contamination of the parental (6-1 X 15-1)F5 stock with subgroup A ALV (see page 44).

Stress

An attempt to evaluate the significance of stress as a source of variation in anti-tumor response was made using dexamethasone and (15I-5 X 6-3)F2 B2/B2 and B15/B15 chickens. Choice of chickens was based on their ALV-free status and availability. Dexamethasone is a synthetic corticosteroid with approximately 25 times the anti-inflammatory potency of hydrocortisone (Haynes and Larner 1975). Under the assumption that at least some of the immunosuppressive effects of stress result from increased blood levels of corticosteroids (Monjan and

Table 7. Segregating B genotypes, ALV status of dams, number of hatches, and number of chickens inoculated with percent tumor induction, by virus, for each F2 population used for evaluating the specificity of anti-Rous sarcoma response.

F2 population	Segregating B genotypes	ALV status of dams	No. of hatches	Number of chickens inoculated and percent tumor induction, by virus: RSV-1	RSV-2	RSV-49
(6-1 x 15-1)F2	2/2,2/5,5/5	positive (approx. 70%)	1	100 (62%)	97 (100%)	97 (75%)
(15I-5 x 6-3)F2	2/2,2/15,15/15	negative	4	109 (100%)	107 (97%)	106 (77%)
(100 x 105)F2	2/2,2/23,23/23	negative	2	40 (100%)	37 (76%)	39 (100%)
{(6-1 x 15-1)F5 x 105}F2	5/5,5/24,24/24	positive (approx. 50%)	2	70 (99%)	64 (78%)	79 (57%)

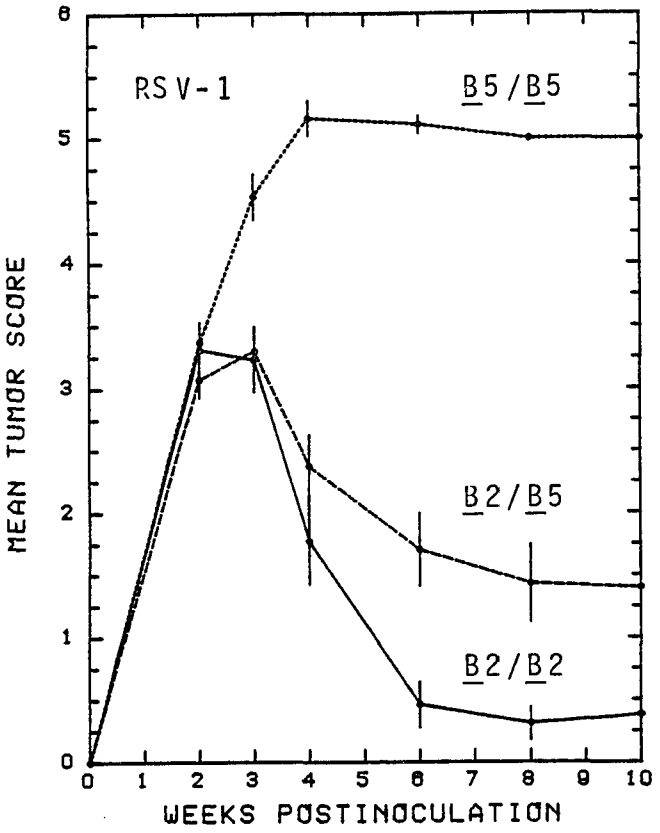

Figure 7A

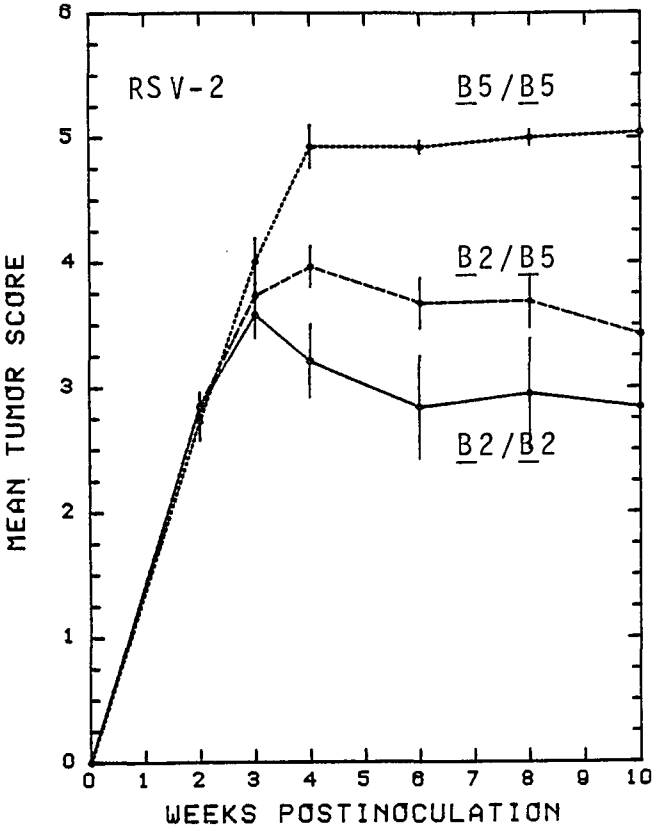

Figure 7B

Figures 7A, B, and C.

Mean tumor scores by weeks postinoculation for (6-1 X 15-1)F2 chickens challenged in the left wingweb with RSV-1 (Fig. 7A), RSV-2 (Fig. 7B), or RSV-49 (Fig. 7C). B2/B2, B2/B5, and B5/B5 chickens are represented by solid, dashed, and dotted lines, respectively. Standard errors are indicated by vertical lines.

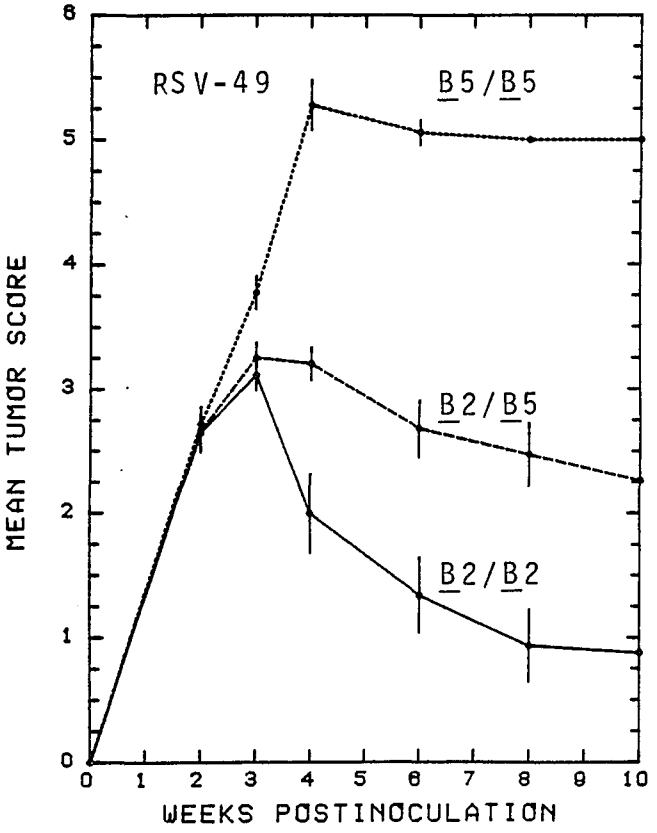

Figure 7C

Table 8A. Analysis of variance of TPI's of (6-1 x 15-1)F2 chickens challenged with RSV-1, RSV-2, or RSV-49

Sources of variation	d.f.	Mean squares
Main effects		
Genotype (G)	2	17.268*
Sex (S)	1	0.533
Virus (V)	2	74.235*
Interactions		
G x S	2	0.197
G x V	4	5.031*
S x V	2	0.743
G x S x V	4	0.479
Residual	214	0.838

*Probability of associated F $\leq$ 0.05.

Table 8B. Least squares mean TPI's by B genotype and virus for (6-1 x 15-1)F2 chickens.

B genotype	Mean TPI, by virus: RSV-1	RSV-2	RSV-49
B2/B2	2.1	3.7	2.4
B2/B5	2.7	4.1	3.5
B5/B5	5.0	5.0	5.0

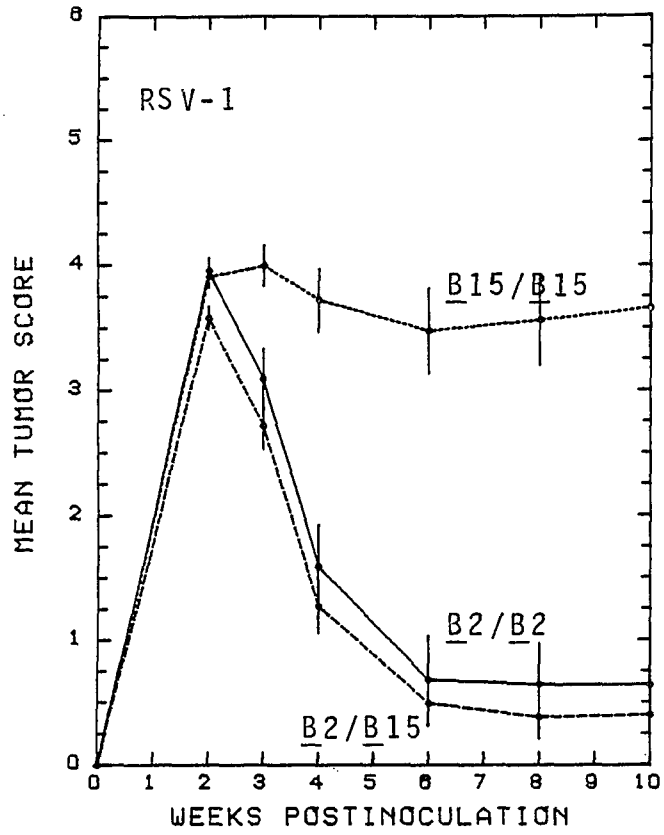

Figure 8A

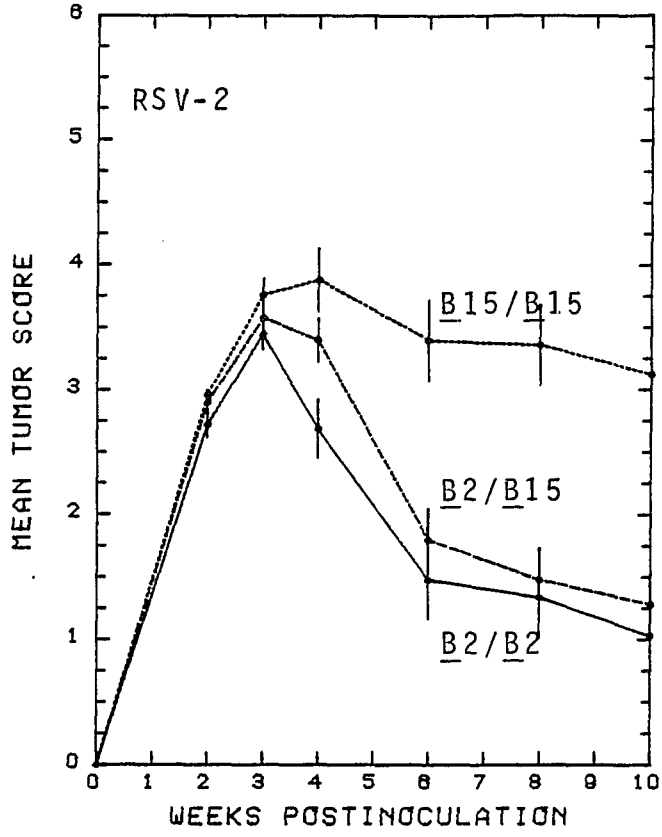

Figure 8B

Figures 8A, B, and C.

Mean tumor scores by weeks postinoculation for (15I-5 X 6-3)F2 chickens challenged in the left wing-web with RSV-1 (Fig. 8A), RSV-2 (Fig. 8B), or RSV-49 (Fig. 8C). B2/B2, B2/B15, and B15/B15 chickens are represented by solid, dashed, and dotted lines, respectively. Standard errors are indicated by vertical lines.

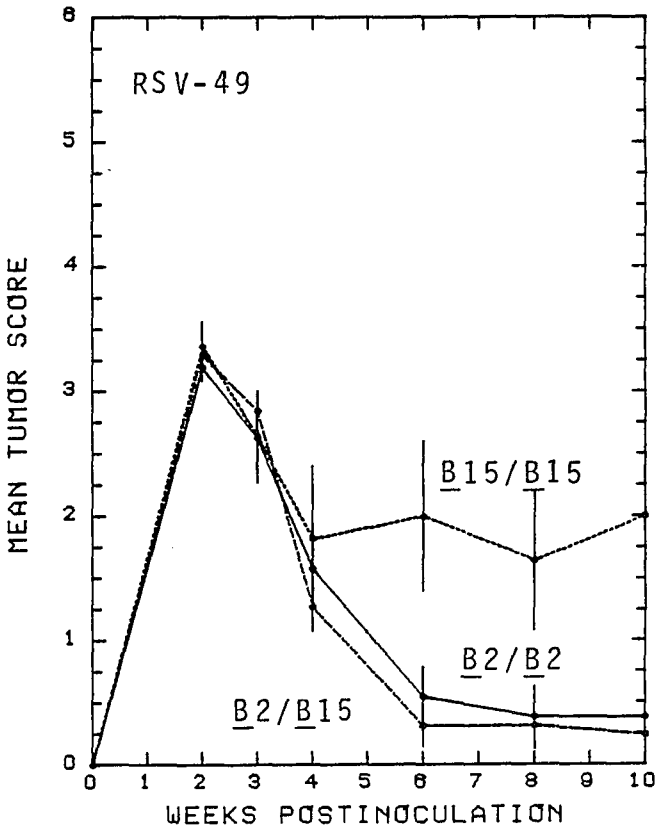

Figure 8C

Table 9A. Analysis of variance of TPI's from (15I-5 x 6-3)F2 chickens challenged with RSV-1, RSV-2, or RSV-49

Sources of variation	d.f.	Mean squares
Main effects		
Genotype (G)	2	43.815*
Sex (S)	1	0.159
Virus (V)	2	12.333*
Hatch	3	5.372*
Two-way interactions		
G x V	4	4.732*
G x S	2	0.093
V x S	2	4.553*
Residual	278	0.928

*Probability of associated F $\leq$ 0.05.

Table 9B. Least squares mean TPI's by B genotype and virus for (15I-5 x 6-3)F2 chickens.

B genotype	Mean TPI, by virus: RSV-1	RSV-2	RSV-49
B2/B2	2.1	2.9	2.1
B2/B15	1.9	3.0	1.9
B15/B15	4.1	3.8	3.2

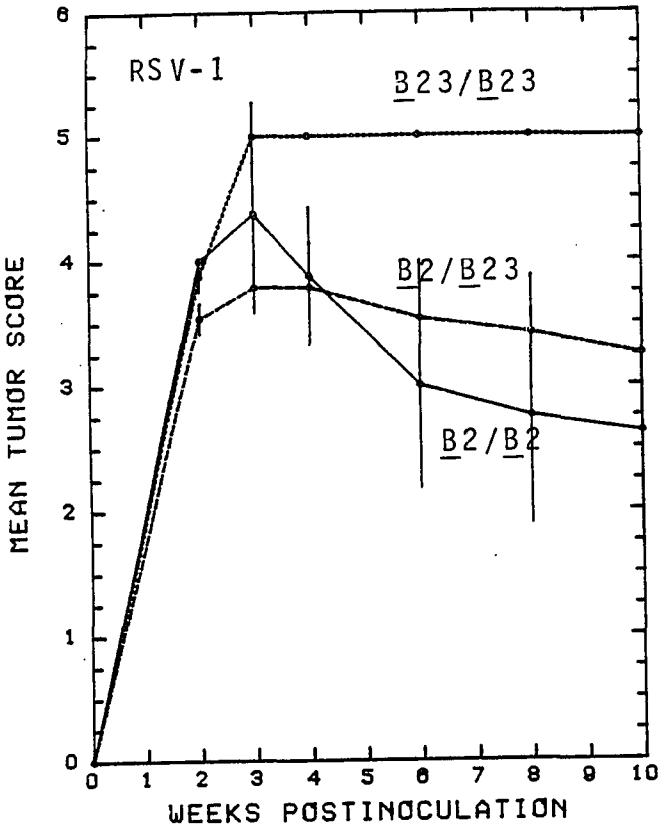

Figure 9A

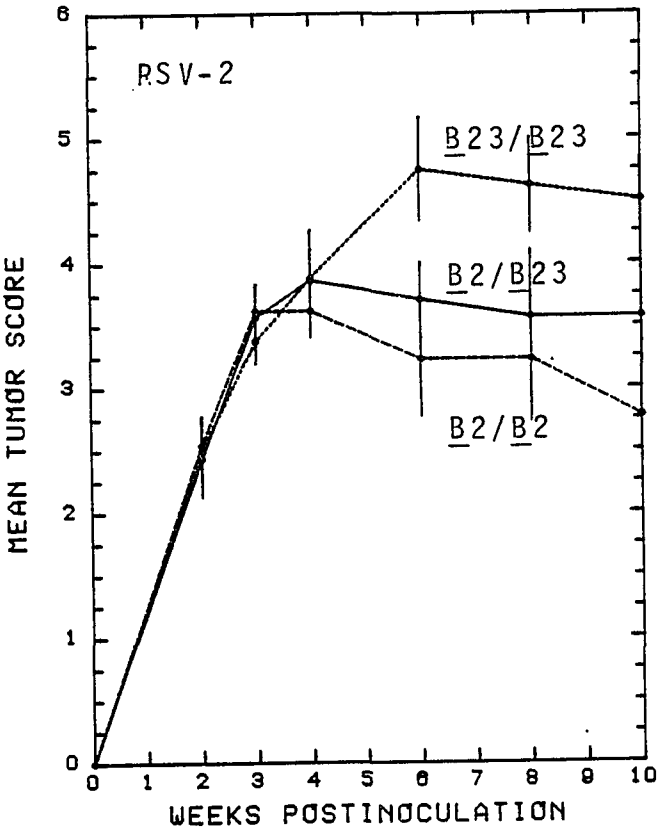

Figure 9B

Figures 9A, B, and C.

Mean tumor scores by weeks postinoculation for (100 X B23/B23 105)F2 chickens challenged in the left wingweb with RSV-1 (Fig. 9A), RSV-2 (Fig. 9B), or RSV-49 (Fig. 9C). B2/B2, B2/B23, and B23/B23 chickens are represented by solid, dashed, and dotted lines, respectively. Standard errors are indicated by vertical lines.

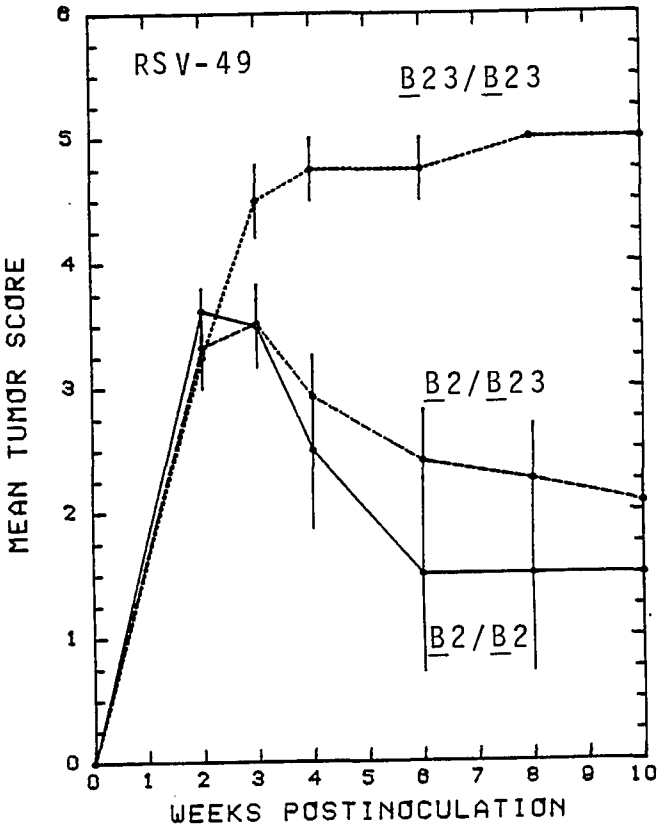

Figure 9C

Table 10A. Analysis of variance of TPI's of (100 x B23/B23 105)F2 chickens challenged with RSV-1, RSV-2, or RSV-49.

Sources of variation	d.f.	Mean squares
Genotype	2	9.597*
Sex	1	0.061
Virus	2	1.743
Hatch	1	0.686
Residual	87	1.440

*Probability of associated F $\leq$ 0.05.

Interactions were not found to be significant, and thus were not included in the table.

Table 10B. Distribution of B2/B2 (100 x B23/B23 105)F2 chickens to TPI, by virus.

Virus	Distribution of chickens to TPI according to virus:				
	1	2	3	4	5
RSV-1	--	2	2	--	4
RSV-2	--	--	1	3	3
RSV-49	--	4	2	--	2

Table 11. Distribution to TPI by genotype and virus for {(6-1 x 15-1)F5 x B24/B24 105} F2 chickens challenged with RSV-1, RSV-2, or RSV-49

B genotype	Virus	Distribution of chickens to TPI according to B genotype and challenge virus: 1	2	3	4	5
B5/B5	RSV-1	--	--	--	--	17
	RSV-2	--	--	--	2	8
	RSV-49	--	--	--	1	11
B5/B24	RSV-1	--	--	--	--	37
	RSV-2	--	--	--	5	15
	RSV-49	--	--	--	1	18
B24/B24	RSV-1	--	--	1	--	14
	RSV-2	--	--	1	3	16
	RSV-49	--	--	1	3	10

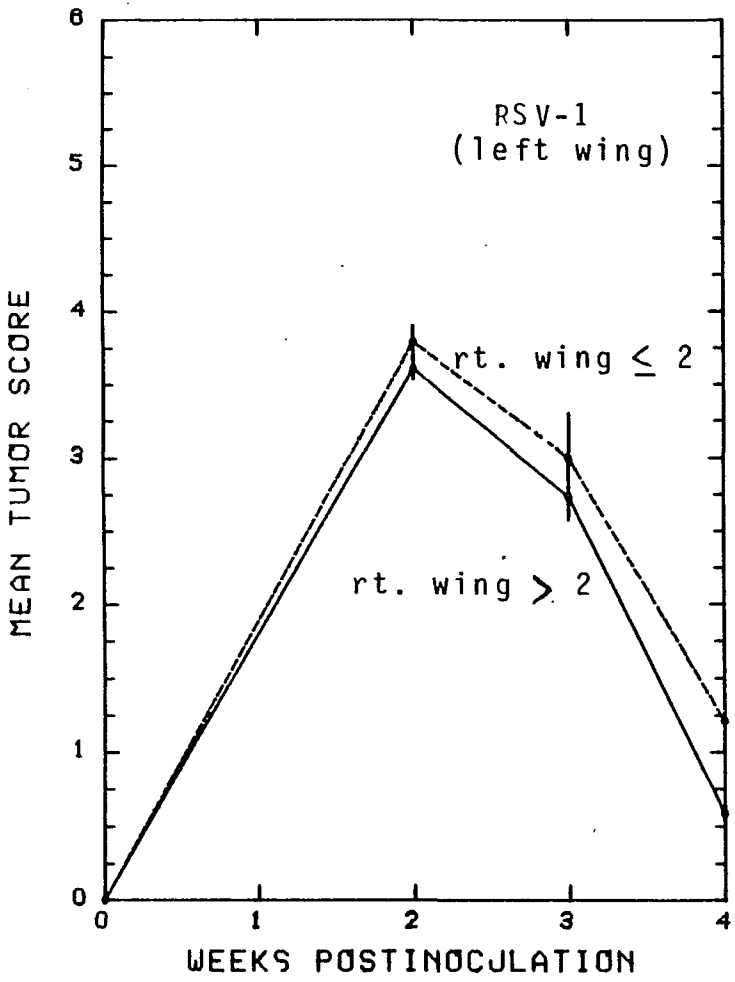

Figure 10A

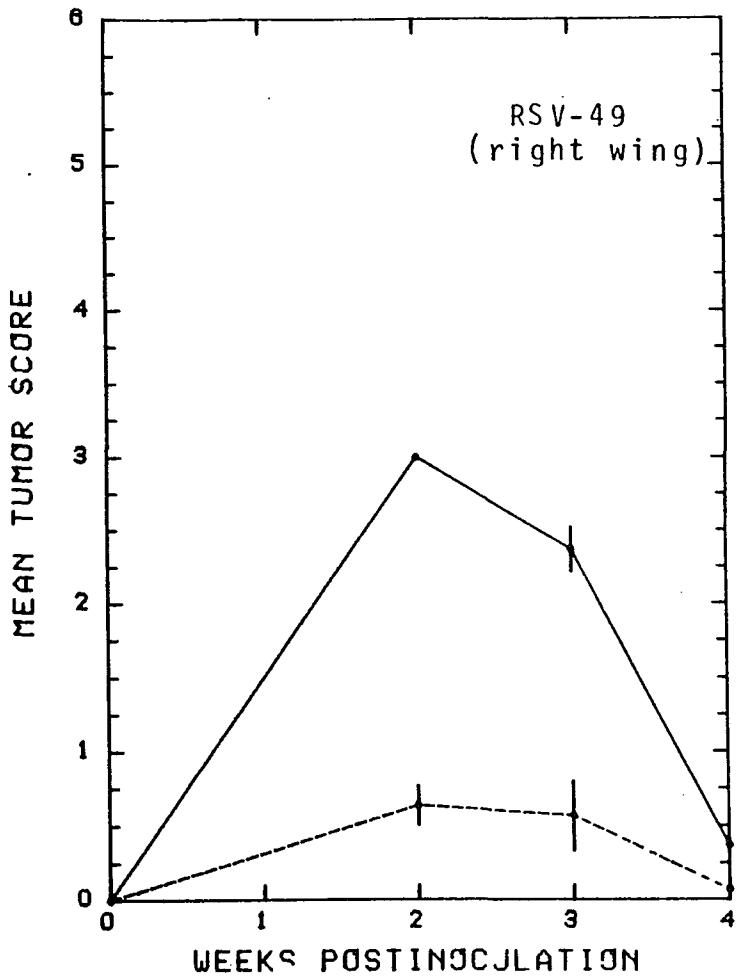

Figure 10B

Figures 10A and B.

Mean left (Fig. 10A) and right (Fig. 10B) wing tumor scores by weeks post-inoculation for B2/B2 (6-1 X 15-1)F2 chickens challenged in the left wingweb with RSV-1 and in the right with RSV-49. Dashed lines represent chickens with small (size 2 or less) RSV-49 induced tumors at 2 weeks PI. Chickens with larger RSV-49 induced tumors (greater than size 2) at 2 weeks PI are represented by the solid lines. Standard errors are indicated by the vertical lines.

Collector 1977, Eskola et al. 1978), B2/B2 and B15/B15 chickens were given intramuscular (IM) injections of dexamethasone prior to and following left wingweb challenge with approximately 1200 PFU of RSV-1. Injections of dexamethasone (0.6 mg/chicken) were given the day of RSV challenge and on 3, 6, 10, 14, and 21 days PI. A 2 mg dose of dexamethasone was given on the 24th day PI. For perspective, the daily anti-inflammatory dosage for a horse is 2.5 to 5 mg IM (McDonald 1969). There were ten B2/B2 and fourteen B15/B15 dexamethasone treated chickens as well as seven B2/B2 and eleven B15/B15 controls (no dexamethasone injections). Mean tumor scores and TPI's were obtained for both treatment and controls. Mean tumor scores by weeks postinoculation are provided in Figures 11A and B (page 73). Treatment and control groups do not appear to differ significantly. If stress was applied, it was not sufficiently immunosuppressive to influence anti-tumor response.

Interaction of MHC Haplotypes in Relation to Rous Sarcoma Regression

Six B genotypes (three B haplotypes) of noninbred line UNH 105 were characterized for ability to respond to RSV-1 induced sarcomas. The genotypes were as follows: B23/B23, B24/B24, B26/B26, B23/B24, B23/B26, and B24/B26. A variety of mating types were used, including some producing only one

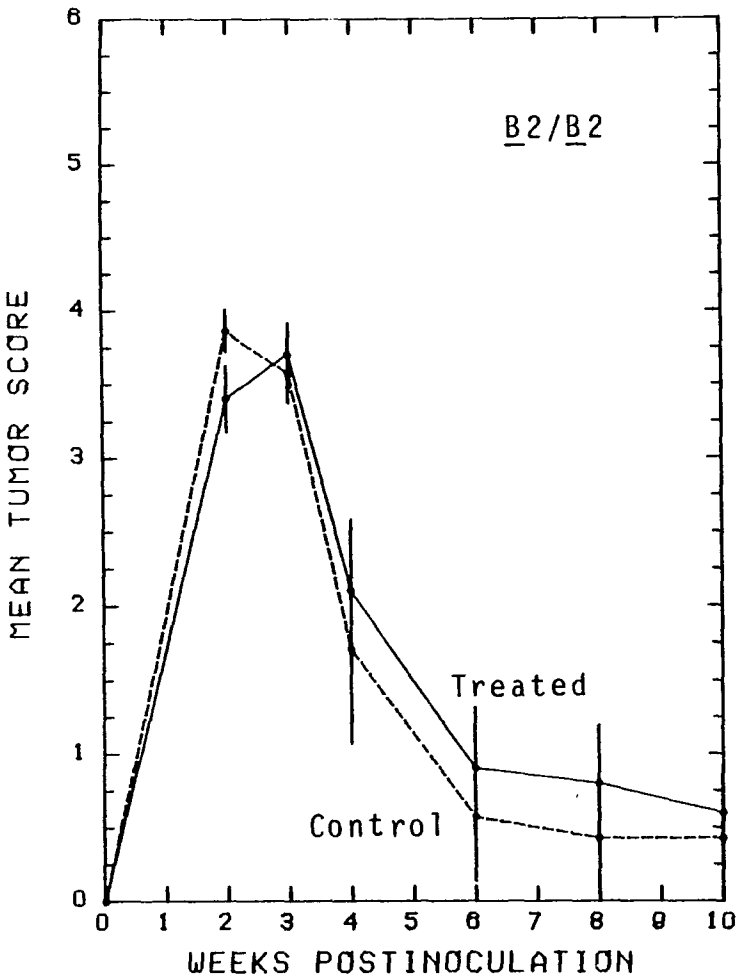

Figure 11A

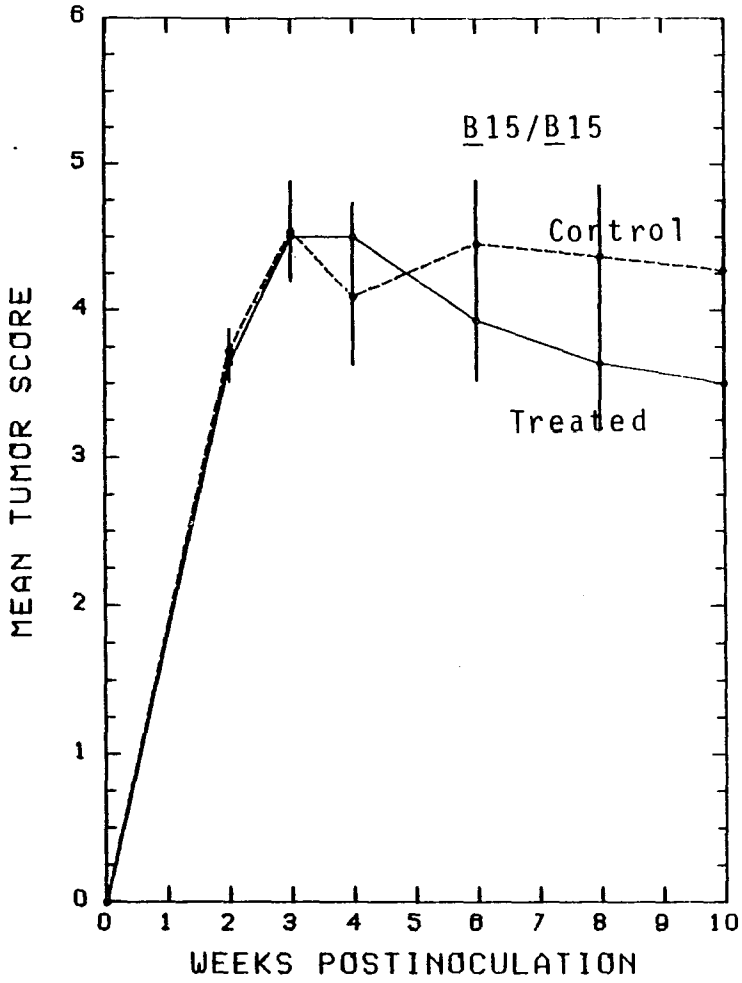

Figure 11B

Figures 11A and B.
Mean tumor scores by weeks postinoculation for B2/B2 (Fig. 11A) and B15/B15 (Fig. 11B) (15I-5 X 6-3)F2 chickens challenged in the left wingweb with RSV-1. Control and dexamethasone treated chickens are represented by dashed and solid lines, respectively. Standard errors are indicated by vertical lines.

B genotype. Six-week-old chickens were inoculated in the left wingweb with approximately 20 PFU of RSV-1. This dosage of RSV-1 was less than that used in the previously described studies. (Using higher doses in this line results in a very low incidence of tumor regression). Tumors were scored as usual over the ten-week postinoculation period, and TPI's were assigned on the basis of a three index TPI (see Materials and Methods). The three index TPI, a modification of the five index TPI of Collins et al. (1977), was used due to the infrequency of complete regression observed among chickens of line UNH 105. The percentage distribution of chickens to TPI's according to B genotype is given in Table 12A (page 77). In an analysis of variance with TPI as the dependent variable and B genotype , sex, and hatch as independent variables (Table 12B), B genotype was a significant source of variation. Results of a means separation test (modified least significant difference of SPSS subprogram ONEWAY) are given in Table 12C (page 78). The homozygous genotypes B23/B23, B26/B26, and B24/B24 had mean TPI's of 2.0, 2.3, and 2.9 respectively, which differed significantly in all comparisons, B23/B23 having the highest response and B24/B24 the lowest. When the B24 haplotype combined with either the B23 or B26 haplotype, anti-tumor response appeared to be slightly lower than that observed for homozygotes of the respective better responding B haplotype, B23 or B26, suggesting a gene dose effect. Highest anti-tumor response was observed among

B23/B26 heterozygotes, suggesting complementation of the B23 and B26 haplotypes for response to RSV-1 induced sarcomas. Only one third of the B23/B26 heterozygotes were produced from B23/B26 X B23/B26 type matings. B23/B23 and B23/B26 siblings from such matings, although demonstrating the same trend as with the combined data of all mating types, did not differ significantly in their anti-tumor response. Thus the possible influence of a non-MHC genetic or environmental factor cannot be ruled out.

Results suggestive of complementation (allelic and/or non-allelic) were also obtained using line 100 and B23/B23 line 105 chickens and progeny of the reciprocal F1 crosses (Table 13, page 79). There were a total of four hatches. At six weeks of age, chickens were inoculated in the left wingweb with approximately 1200 PFU of RSV-1. Tumors were scored as usual and a five index TPI (see Materials and Methods) was used to evaluate anti-tumor response. (100 X 105)F1 chickens (B2/B23) had higher anti-tumor responses (lower TPI's) than those observed with either parental type. Such results could be explained by both MHC and non-MHC genetic effects. The chickens used were not from ALV-free dams, a fairly high incidence of ALV contamination being found among line 100 dams and a low incidence among line 105 dams. Therefore, the high anti-tumor response associated with progeny from the 100 X 105 mating type could have resulted from the favorable combination of the B2 haplotype from line 100 and ALV-free line 105 dams. As previously

noted (page 51), a comparison of F1 progeny from ALV-negative and -positive dams clearly indicated that the ALV status of the dams did significantly influence the anti-tumor response of the progeny (see Table 5, page 53).

A (100 X B23/B23 105)F2 population (see page 43) produced from ALV-negative dams provided no evidence of MHC complementation. B2/B23 responses to RSV-1, RSV-2, and RSV-49 induced sarcomas were slightly lower than those associated with B2/B2 (Figures 9A, B, and C; page 68). On the other hand, evidence of complementation presumably resulting from the favorable combination of the B2 haplotype of line 100 with the non-MHC background of line 105 was obtained in the comparisons of F2 and backcross progenies of lines 100 and 105 (Figures 5A, B, and C; page 49). Highest anti-tumor responses were associated with B2/B23 progeny from the backcross to line 105. All dams were ALV-negative.

Attempts to Localize the Non-MHC Genetic Influence on Rous Sarcoma Regression

Endogenous Virus, RAV-0

Line 7-2 is homozygous for ev-2, a locus which codes for the nondefective endogenous ALV, RAV-0 (Astrin et al. 1980). Lines 6-1 and 6-3 lack this endogenous virus, but they do have ev-3, a locus coding for a defective endogenous virus that produces virus envelope (Astrin et al. 1979; Robinson, personal communication). Although

Table 12A. Percentage distribution of line UNH 105 chickens to TPI's, by B genotype, for tumors induced by RSV-1.

B genotype	Percentage distribution to tumor profile index: 1	2	3
23/26	54	23	23
23/23	34	32	34
23/24	17	39	44
26/26	26	15	59
24/26	14	21	65
24/24	0	13	87

Table 12B. Analysis of variance of TPI's of line 105 chickens challenged with RSV-1.

Sources of variation	d.f.	Mean squares
Genotype	5	15.083*
Sex	1	0.000
Hatch	5	1.374*
Residual	733	0.612

*Probability of associated $F \leq 0.05$.

Interactions were not found to be significant, and thus were not included in the table. In a second analysis in which interaction effects were pooled with the residual, hatch effect was not significant (see Brown et al., 1982).

Table 12C. Mean TPI's by B genotype together with a means separation test for line UNH 105 chickens challenged with RSV-1.

	B genotype:					
	23/26	23/23	23/24	26/26	24/26	24/24
Mean TPI	1.7^{a}*	2.0^{b}	2.3^{bc}	2.3^{c}	2.5^{c}	2.9^{d}
Number/genotype	145	176	88	181	133	76

*Means having no superscripts in common are significantly different, $P \leq 0.05$.

Table 13. Mean tumor scores at two weeks PI and mean TPI's according to mating type for line 100, B23/B23 line 105 and (100 x 105) reciprocal F1 chickens challenged in the left wingweb with RSV-1.

Mating type (Male)	(Female)	B genotype of progeny	Mean tumor score**	Number of chickens	Mean TPI	Number of chickens
100	x 100	2/2	3.25^{b}*	55	3.49^{b}*	55
100	x 105	2/23	3.64^{a}	37	2.80^{c}	40
105	x 100	2/23	3.47^{ab}	96	3.32^{bc}	96
105	x 105	23/23	3.80^{a}	125	4.37^{a}	126

*Means having no superscripts in common differ significantly, $P \leq 0.05$.

**Mean tumor scores at two weeks PI (birds without tumors excluded, including 4 birds which developed tumors by the third week and were assigned TPI's.

presence of the defective endogenous virus encoded by ev-3 does not result in progressive sarcoma growth in lines 6-1 and 6-3, it is conceivable that RAV-0, a nondefective endogenous virus capable of replication, may influence response (perhaps by tolerizing the host to a non-virion antigen associated with ALV replication). The possible influence of the ev-2 locus was evaluated using lines 6-1 and 7-2. Line 6-1 dams were inseminated with the pooled semen from 6-1 and 7-2 males. Thus both 6-1 and (7-2 X 6-1)F1 chickens were produced simultaneously by the same dams thereby reducing the influence of maternal effects on results. Progeny were blood typed for E genotype to differentiate 6-1 chickens from (7-2 X 6-1)F1's. Six-week-old chickens were inoculated in the left wingweb with approximately 1200 PFU of RSV-1. The distribution of TPI's to 6-1 and (7-2 X 6-1)F1 chickens is provided in Table 14 (page 83). Sample size is too small to differentiate between the two groups. Of significance, however, is the observation that only 2 of 12 (7-2 X 6-1)F1 progeny demonstrated progressive tumor growth (TPI of 4 or 5) which differs considerably from the high incidence of progression observed among (15I-5 X 7-2)F1 chickens (page 39). Since both (7-2 X 6-1)F1 and (15I-5 X 7-2)F1 chickens are heterozygous for presence of ev-2, yet another variable must have been responsible for the high incidence of progression among (15I-5 X 7-2)F1's.

Line K28 is a noninbred line with only one endogenous

virus, encoded by ev-1 (Robinson et al. 1980). This endogenous virus, present in nearly all White Leghorn lines tested so far, produces neither intact virus nor envelope antigens (Astrin and Robinson 1979). The cells of this line of chickens, free of interference from subgroup E virus envelope antigens, have the capacity for efficient replication of subgroup E viruses such as RAV-0. For this reason, line K28 chickens were used in an attempt to immunize against Rous sarcoma challenge using the endogenous virus, RAV-0. Chickens were divided into four treatment groups each of which received three biweekly inoculations prior to challenge with RSV-1 (approximately 1200 PFU) in the left wingweb and RSV-2 (approximately 7500 PFU) in the right wingweb at eight weeks of age. The biweekly inoculations (given at 16, 28, and 42 days after hatching) for the four treatment groups were as follows:

Group 1 - RAV-0 stock, 1 ml IM

Group 2 - Dulbeccos MEM, 1 ml IM (Control)

Group 3 - RAV-1, 1:100 dil. of stock (1 ml IM)

Group 4 - 6-1 embryo tissue (11 day), a source of subgroup E virus envelope (5% suspension, 1 ml subcut.).

If RAV-0 replicating cells or chf(+) cells bear surface antigens similar to those involved in anti-Rous sarcoma response, then immunization with RAV-0 (Treatment group 1) or with chf(+) cells (Treatment group 4) should result in enhanced response to RSV-induced tumors. Immunization with

the exogenous ALV, RAV-1, has been previously shown to result in enhanced anti-Rous sarcoma response (Meyers *et al*. 1972).

Mean tumor scores for each treatment group over the ten weeks following inoculation with RSV are shown for RSV-1 and RSV-2 induced sarcomas in Figures 12A and B, respectively (page 84). Tumors which did not develop were not included in the computation of mean tumor scores. The distribution of two week tumor scores to treatment group are provided for RSV-1 and RSV-2 induced tumors in Table 15 (page 85). Considerable variablity is apparent within each treatment group. There is no evidence of enhanced anti-tumor response in chickens immunized with either RAV-0 or with 6-1 embryo tissue. Chickens immunized with RAV-1, on the other hand, demonstrated greater resistance to tumor induction with both subgroup A and subgroup B RSV, indicating that such resistance was not the result of virus receptor interference (Interference by RAV-1 would be limited to subgroup A viruses.).

The *E* Blood-Group Locus

Progressor lines 7-2, 100, and 15I-5 are homozygous for the *E*5 haplotype, whereas regressor lines 6-1 and 6-3 are homozygous for *E*7. The (15I-5 X 6-3)F1 X 7-2 chickens used for evaluating the *B*2 haplotype of line 7-2 (page 41) provided an opportunity for determining whether or not response to Rous sarcoma was associated with the *E*

Table 14. Distribution of TPI's to 6-1 and (7-2 x 6-1) F1 chickens following challenge with RSV-1

Mating Type (Male) (Female)	Distribution of chickens to TPI according to mating type				
	1	2	3	4	5
7_2 x 6_1	1	4	5	1	1
6_1 x 6_1	1	5	1	0	0

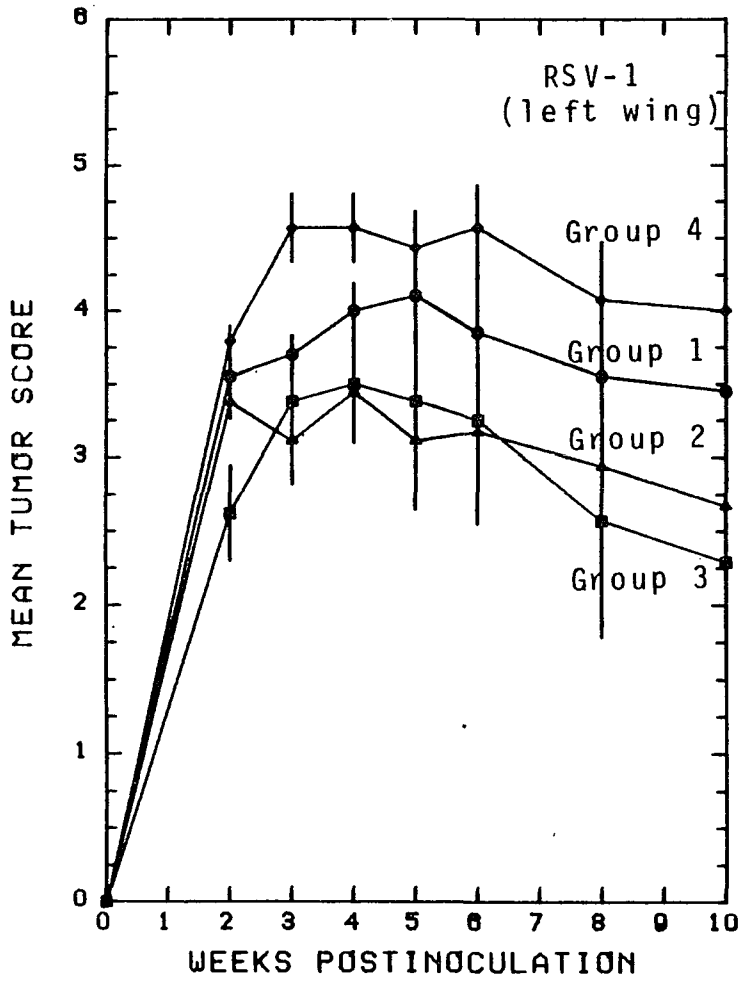

Figure 12A

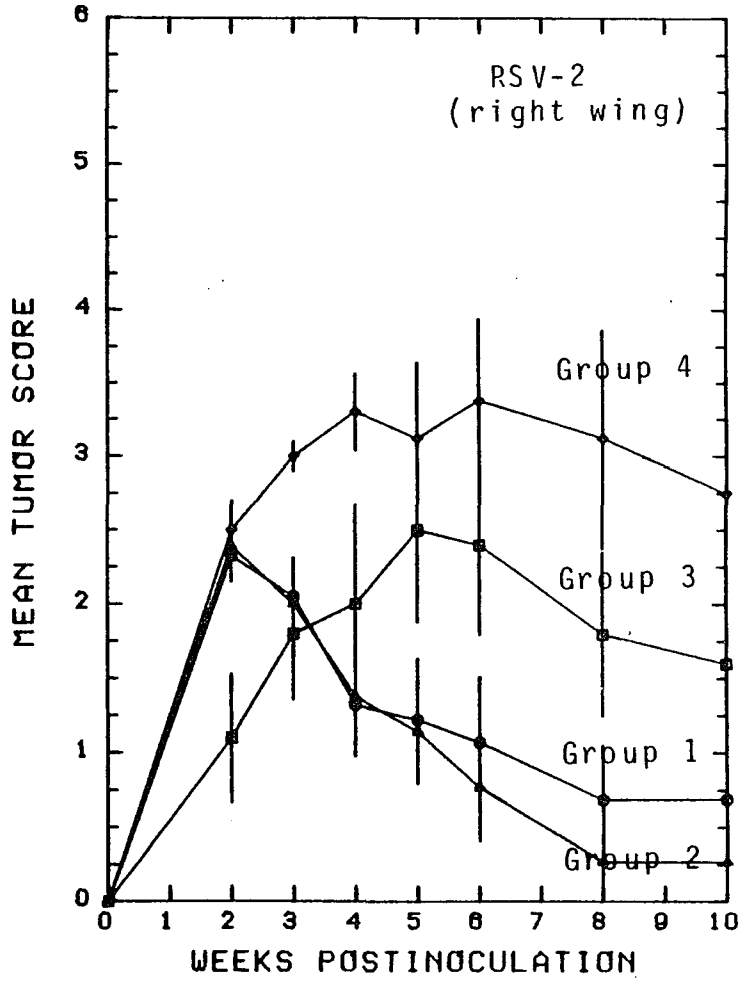

Figure 12B

Figures 12A and B.
Mean tumor scores by weeks postinoculation for K28 chickens challenged in the left wingweb with RSV-1 (Fig. 12A) and in the right (Fig. 12B) with RSV-2. The legend for the three treatment groups plus controls is as follows:

Group 1 - RAV-0 immunized
Group 2 - controls
Group 3 - RAV-1 immunized
Group 4 - immunized with line 6-1 embryo tissue.

Table 15. Distribution of tumor scores (2 weeks PI), according to treatment and virus, from K28 chickens challenged in the left wing with RSV-1 and in the right with RSV-2.

Treatment group	Distribution of tumor scores (2 weeks PI) according to treatment: 0	1	2	3	4
			RSV-1		
RAV-0 immunized	--	--	--	9	11
Controls	--	--	--	12	7
RAV-1 immunized	6	1	2	4	1
Immunized with line 6-1 embryo tissue	--	--	--	3	11
			RSV-2		
RAV-0 immunized	1	3	7	9	--
Controls	1	4	3	11	--
RAV-1 immunized	9	2	--	3	--
Immunized with line 6-1 embryo tissue	--	2	3	9	--

blood-group locus (The segregating B2/B2 and B2/B15 genotypes in that experiment did not differ.). The decision to evaluate a possible E locus effect was made in retrospect while the experiment (page 41) was in progress. Four chickens had died. The remaining 31 chickens were blood typed for E alloantigens. There were 18 E5/E5 homozygotes and 13 E5/E7 heterozygotes. Their responses to RSV-1 and RSV-49 induced sarcomas are diagramatically represented in Figures 13A and B, respectively (page 87). There is no indication of depressed anti-sarcoma response among the E5/E5 homozygotes. These results confirm previous observations with (6-3 X 100)F2 chickens segregating at the E locus (Collins, unpublished data).

MHC vs Non-MHC Influences on Metastasis

In the study characterizing six B genotypes of line 105 for response to RSV-1 induced tumors (page 72), the overall incidence of histologically confirmed metastasis among line 105 chickens with terminal tumors was approximately 27%. B genotype did not appear to significantly influence the frequency of metastasis (Table 16, page 90). This incidence, however, was substantially lower than that for gross metastasis previously observed among B5/B5 (6-1 X 15-1)F2 chickens (Collins *et al.* 1977). Neither length of time from inoculation to death (Table 17A, page 91) nor sex (Table 17B) appeared to significantly influence the

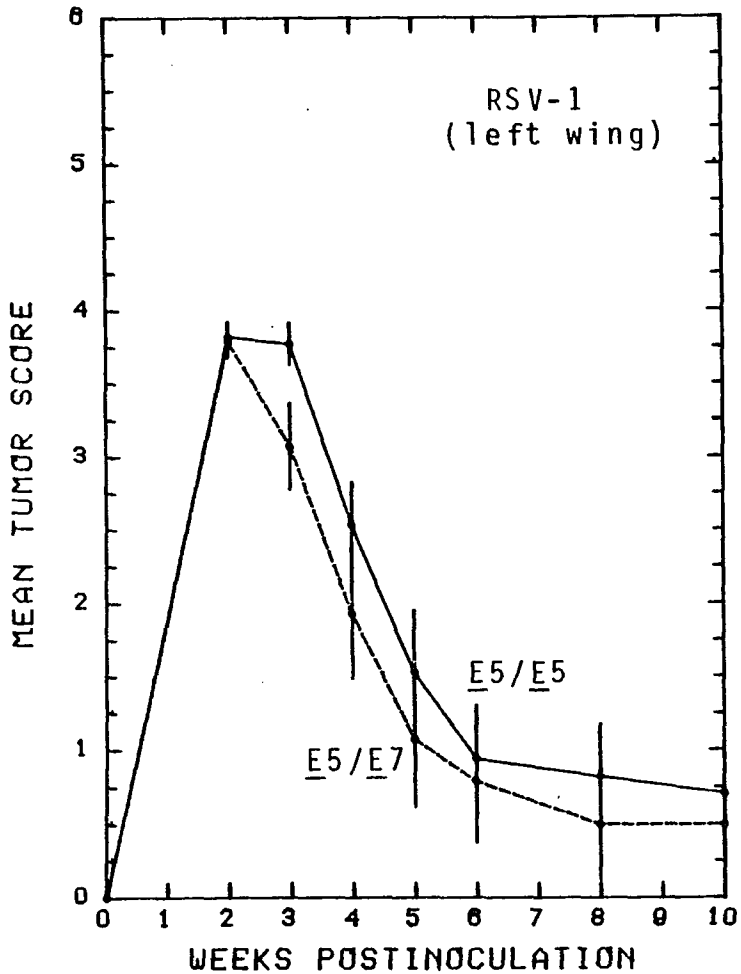

Figure 13A

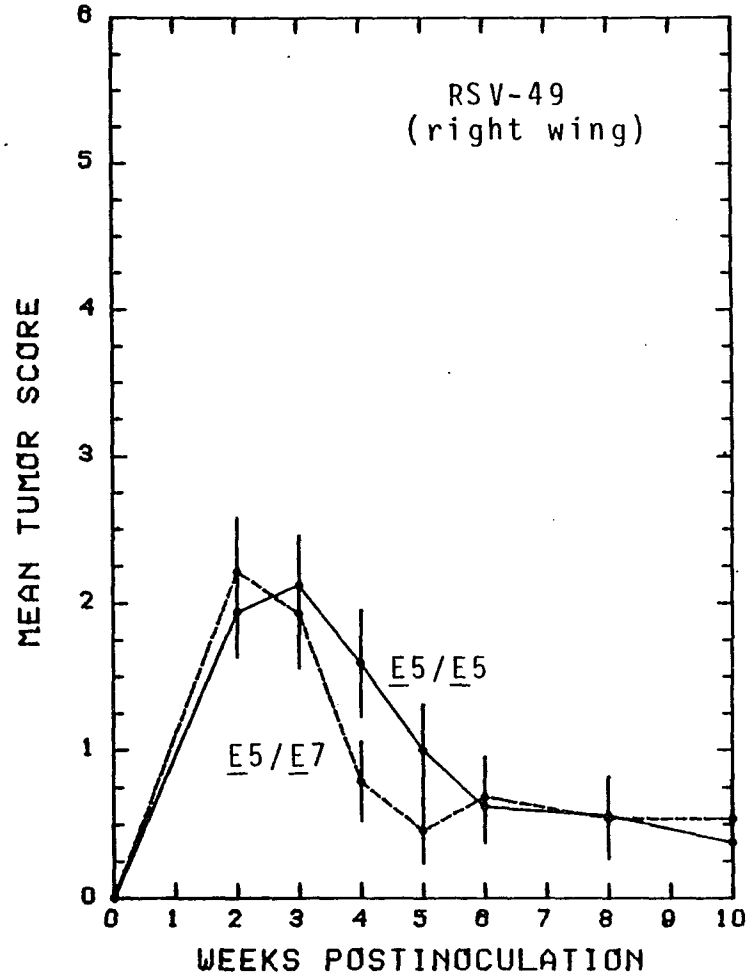

Figure 13B

Figures 13A and B.

Mean tumor scores by weeks postinoculation for (15I-5 X 6-3)F1 X 7-2 chickens challenged in the left wingweb with RSV-1 (Fig. 12A) and in the right with RSV-49 (Fig. 12B). E5/E5 and E5/E7 chickens are represented by solid and dashed lines, respectively. Standard errors are indicated by vertical lines.

development of metastatic lesions.

In a further attempt to discern possible genetic influences on metastasis, reciprocal crosses were made between B5/B5 (6-1 X 15-1)F5 and B24/B24 line 105 chickens. Progeny of these "F1's", as well as progeny of the parental stocks, were challenged in the left wingweb at six weeks of age with approximately 1200 PFU of RSV-1. Table 18 (page 92) includes the combined data of four hatches. Sixty six percent of B5/B5 (6-1 X 15-1)F6 chickens with terminal tumors had at least one histologically confirmed metastatic lesion. The incidence of metastasis in terminal cases from the other three mating types was 24% or less. The reciprocal "F1" mating types produced similar results; thus maternal effects would not appear to have accounted for the higher incidence of metastasis observed among B5/B5 (6-1 X 15-1)F6 chickens. Results of chi square analysis (Table 19A, page 93) suggest that mating type significantly influenced susceptibility to metastasis.

Among F2 progeny resulting from the *inter se* matings of (B5/B5 (6-1 X 15-1)F5 X B24/B24 105)F1's (see page 62), the overall incidence of histologically confirmed metastatic lesions associated with terminal RSV-1, RSV-2, or RSV-49 induced tumors was low (19%). The distribution of B5/B5, B5/B24, and B24/B24 chickens to presence or absence of confirmed metastasis is given in Table 19B (page 93). In this F2 population, the B5/B5 genotype is not associated with a high incidence of metastasis. Therefore the low

incidence of metastasis associated with line 105 and its crosses appears to be the result of non-MHC genetic influences.

Table 16. Distribution of line 105 chickens to presence or absence of metastasis according to B genotype.

Metastasis*	B genotype					
	23/26	23/23	23/24	26/26	24/26	24/24
Yes	8	15	8	23	20	19
No	20	33	25	79	58	40

$X^2 = 2.51$; $P > 0.05$.

*All chickens died of RSV-1 induced sarcomas. Upon necropsy, any suspect lesions were sectioned and prepared for histological examination. Six cases are included, however, in which presence of metastasis was determined by gross examination alone.

Table 17A. Distribution of line 105 chickens to presence or absence of histologically confirmed metastasis according to length of time from inoculation with RSV-1 to death.

Confirmed metastasis	Days (PI) to death:		
	0-30	31-40	40-70
Yes	31	30	32
No	104	83	68

$X^2 = 2.40$; $P > 0.05$.

Categories were chosen to give approximately equal numbers in each.

Table 17B. Distribution of line 105 chickens to presence or absence of histologically confirmed metastasis according to sex.

Confirmed metastasis	Sex:	
	Male	Female
Yes	50	43
No	121	130

$X^2 = 0.63$; $P > 0.05$.

Four birds were deleted from the analysis because sex was unknown.

Table 18. Frequency of metastasis by mating type for B5/B5 (6-1 x 15-1)F6 and B24/B24 line 105 chickens and progeny of the reciprocal intercrosses.

Mating type (Male)	(Female)	B genotype of progeny	Number inoculated	Tumor developed (No.)	(%)	No. of terminal cases	Mean days (PI) until death	Metastasis* (%)
F5	x F5	5/5	106	82	77	82	26.5	66
F5	x 105	5/24	40	29	72	26	31.4	19
105	x F5	5/24	71	46	65	45	23.4	24
105	x 105	24/24	93	88	95	81	28.4	12

*Percent metastasis represents the percentage of terminal cases with at least one histologically confirmed metastatic lesion. Tumors were induced by RSV-1.

Table 19A. Distribution by mating type of B5/B5 (6-1 x 15-1)F6 and B24/B24 line 105 chickens and progeny of the reciprocal intercrosses to presence or absence of histologically confirmed metastasis of RSV-1 induced sarcomas.

Mating type (Male)	(Female)	Metastasis	No metastasis
F5	F5	54	28
F5	105	5	21
105	F5	11	34
105	105	10	71

$X^2 = 58.2$; $P \leq 0.05$.

Table 19B. Distribution of {B5/B5 (6-1 x 15-1)F5 x B24/B24 105} F2's to presence or absence of metastasis according to B genotype.

Confirmed metastasis*	B genotype: 5/5	5/24	24/24
Yes	5	12	10
No	30	57	28

$X^2 = 1.94$; $P > 0.05$.

*Metastatic lesions were confirmed histlogically. All cases represent terminal RSV-1, RSV-2, or RSV-49 induced tumors.

V. DISCUSSION

Influence of Genetic Background on Anti-Sarcoma Response

RPRL inbred lines 6-1, 6-3, 7-2, and 100, all homozygous for the B2 haplotype, have differed greatly in response to RSV-induced sarcomas (Collins et al. 1980). Results of studies included in this dissertation indicate that the observed variation between lines was the result of non-MHC influences rather than undetected differences within the MHC.

(15I-5 X 7-2)F1 chickens (B2/B15) had progressive tumor growth after challenge with RSV-1 in the left wing and RSV-49 in the right, whereas (15I-5 X 6-1)F1 chickens (B2/B15) rapidly regressed both tumors (Fig. 1A and B; page 45). These results confirmed the findings of Marks et al. (1979) that the incidence of tumor regression associated with line 7-2 was low compared to that associated with lines 6-1 and 6-3. However the cause of the variation between the F1's, MHC or non-MHC, could not be determined since both MHC and non-MHC variation could adequately explain the results.

That low response associated with line 7-2 was the result of a non-MHC influence became apparent after RSV challenge of (15I-5 X 6-3)F1 X 7-2 chickens. Both B2/B2 and B2/B15 chickens of this cross regressed RSV-1 and RSV-49

induced sarcomas (Fig. 2A and B; page 46). B2/B15 chickens received their B2 haplotype from line 7-2, and thus were identical at the MHC to the (15I-5 X 7-2)F1's of the previous experiment. Since the B15 haplotype was associated with progressive tumor growth, as determined by (15I-5 X 6-3)F2 data (Fig. 8A, B, and C; page 66), the high incidence of regression observed with B2/B15 [(15I-5 X 6-3)F1 X 7-2] chickens was attributed to the B2 haplotype of line 7-2. Thus a non-MHC influence associated with line 7-2 appeared to be capable of suppressing response against RSV-induced sarcomas.

Assuming involvement of a single non-MHC gene (dominant for high response in line 6-1 or recessive for low response in lines 7-2 and 15I-5), approximately 50% of the (15I-5 X 6-3)F1 X 7-2 chickens should have been characterized by progressive tumor growth. Only 15% were progressors. 6-1 X (15I-5 X 6-3)F1 and (15I-5 X 6-3)F1 X 6-3 chickens receiving the same RSV inoculations as the above (15I-5 X 6-3)F1 X 7-2 chickens were characterized by tumor regression more rapid than that observed with (15I-5 X 6-3)F1 X 7-2 (Fig. 4A and B; page 48). These results do not support the hypothesis of involvement of only one non-MHC gene, but instead favor the involvement of multiple non-MHC loci and/or an interaction between an environmental variable and a non-MHC genotype.

The relative importance of non-MHC genetic background for anti-Rous sarcoma response was further evaluated using crosses of lines 100 and 105 carried to backcross and F2

generations. B2/B23 chickens from the F1 X 105 backcross had a high incidence of regression of tumors induced by each of three different subgroups of RSV, whereas B2/B2 and B2/B23 chickens from the F1 X 100 backcross were characterized by progressive tumor growth (Fig. 5A, B, and C; page 49). Since B23/B23 chickens developed progressive tumor growth regardless of associated mating type, regression associated with B2/B23 chickens from the F1 X 105 backcross was attributed to the B2 haplotype from line 100. Therefore a non-MHC influence, in this case associated with line 100, again appeared to suppress anti-Rous sarcoma response. Suppression was generalized since it appeared regardless of RSV subgroup used.

Environmental Influences on Host Response to RSV-Induced Sarcomas

Avian Leukosis Virus (ALV)

Congenital infection with subgroup A ALV resulted in increased resistance to tumor induction with RSV-1 and a higher incidence of tumor progression among (100 X B23/B23 105)F1 progeny (Table 5, page 53). These results were consistent with previous reports (Rubin 1962, Vogt and Ishizaki 1966, Meyers and Qualtiere 1977).

Screening of different lines and crosses maintained at UNH revealed variability between lines in degree of ALV contamination. Lines 7-2 and C, for example, being

genetically resistant to subgroup A ALV, were virtually free of ALV contamination. Lines 15I-5 and 100, on the other hand, being susceptible to subgroup A ALV, had high incidences of ALV contamination. Under conditions in which ALV contamination is not controlled, between line comparisons for anti-Rous sarcoma response could clearly be influenced by variability between lines in degree of ALV contamination. Gebriel et al. (1979), for example, reported considerable variability in the tumor induction period following RSV-1 challenge of high and low responders to the amino acid polymer, GAT. GAT-high responders developed tumors more rapidly and had higher frequencies of tumor regression than GAT-low responders. The ALV status of the experimental chickens was not reported. Delayed tumor induction, low response to RSV-1 induced tumors, and decreased response to GAT could all result from congenital infections with subgroup A ALV.

Eggs from seven of eleven (6-1 X 15-1)F5 dams checked for ALV shedding were found to be positive. Heinzelmann et al. (1981b) using F4 and F5 generation chickens of the 6-1 X 15-1 cross, reported cross-reactivity between the B5 antigen(s) and RSV-induced tumor-associated antigens. Lymphocytes from B2/B2 chickens bearing RSV-induced tumors lysed normal B5/B5 chick embryo fibroblasts (CEF), but not normal B2B2 CEF or normal B24/B24 cells from line 105. In light of known ALV contamination, the observed lysis of "normal" B5/B5 CEF may have been the result of ALV

contamination of these cells.

Marek's Disease

Lines 15I-5, 7-2, and 100 are all susceptible to Marek's disease (MD), whereas lines 6-1 and 6-3 are resistant (Altman and Katz 1979). Since MD virus is immunosuppressive (Jakowski et al. 1970), highly contagious (Calnek and Witter 1978), and capable of suppressing anti-Rous sarcoma response (Calnek et al. 1975), it is conceivable that the low anti-Rous sarcoma responses associated with the MD-susceptible lines may have been a result of environmental exposure to MD (in spite of MD vaccination).

The higher incidence of tumor progression observed in one hatch of (15I-5 X 6-3)F2 chickens (see Table 6A, page 57) was associated with known exposure to MD. Histological examination of bursas from (15I-5 X 7-2)F1 and (15I-5 X 6-3)F1 X 7-2 chickens dying with terminal tumors revealed cystic follicles and lymphocytic infiltration of the interfollicular connective tissue (Fig. 6B, page 56), lesions characteristic of MD (Calnek and Witter 1978).

Vaccination is known to be less effective in genetically susceptible stock and does not prevent superinfection with virulent virus (Powell 1981). In spite of MD vaccination of both replacement and experimental chickens, occasional cases of MD and increased mortality has been observed among MD-susceptible lines (see Table 20, page

101). Among experimental chickens, the incidence of clincial signs or gross lesions associated with MD has been low. Immunosuppression resulting from exposure to MD virus, however, would occur early in the course of the disease, at the time of virus replication in the bursa and thymus (Powell 1981). Thus progressive Rous sarcoma growth resulting from such immunosuppression could occur prior to development of gross MD lesions or clinical signs of MD. It is also conceivable that lyophilized HVT (turkey herpesvirus) used for MD vaccination at UNH, although providing at least partial protection against development of MD tumors, may have failed to protect susceptible chickens against the immunosuppressive effect of MD virus replication in the bursa and thymus.

Examination of the bursa and thymus from RSV-challenged (6-3 X 15-1)F4 chickens revealed that all of seventy-two progressors had severe atrophy of both organs (1/8 to 1/4 normal size), whereas the bursa and thymus of all seventy-nine regressors were normal (Fadly and Bacon 1979). Histological examination of both bursa and thymus from the seventy-two progressor birds revealed atrophy of lymphoid follicles and pronounced connective tissue proliferation. It was not determined whether RSV-induced tumors progressed because of destruction of the host's lymphoid system or whether the destruction was caused by the tumors. The normal bursa of the line 105 chicken dying of progressive sarcoma growth (Fig. 6A, page 56) would suggest that

progressive Rous sarcoma growth among the (6-3 X 15-1)F4's reported by Fadly and Bacon was not the cause but the result of destruction of the host's lymphoid system. Such degenerative changes in the bursa and thymus could result from exposure to MD virus.

Considerable variation in bursal size was found among 16-week-old (6-1 X 15-1)F2 chickens which had not been inoculated with RSV. A high incidence of bursal atrophy was associated with the B5/B5 genotype (Table 21, page 102). Histological evidence for MD involvement was obtained from sections of the atrophied bursas.

Sharma and Burmester (1982) reported that MD vaccination on the 18th day of embryological development resulted in greater resistance to subsequent challenge with MD virus than obtained by conventional MD vaccination at hatching. In an attempt to improve anti-Rous sarcoma response by preventing MD virus-mediated immunosuppression, (15I-5 X 7-2)F1 and (15I-5 X 6-1)F1 chickens were MD vaccinated on the 18th day of incubation rather than at hatching. They were subsequently challenged at six weeks of age with RSV-1 and RSV-49 in the left and right wingwebs, respectively (Brown and DiFronzo, unpublished data). Figures 14A and B (page 103) provide the mean tumor scores for the left and right wing tumors. Responses of (15I-5 X 6-1)F1's was nearly identical to previous findings (see Figures 1A and B, page 45). (15I-5 X 7-2)F1's, however, were characterized by a lower incidence of progressive tumor

Table 20. Mortality data of some recent replacement hatches of chickens MD-vaccinated at hatching, University of New Hampshire Poultry Farm

	Hatch:		No. culled:[b]		No. died:			
	Date	No. chicks[a]	(M)	(F)	(No info.)	(Cause reported)	Mortality[c] (%)	Incidence[d] of MD (%)
6-1	12-2-80	23	3	0	4	1	25	0
	3-24-81	77	22	7	4	0	8	0
15-1	3-24-81	22	6	6	4	3	70	0
	4-7-81	26	3	5	10	5	83	0
	4-21-81	29	9	2	6	8	78	11
	5-5-81	21	5	5	5	2	64	0
15I-5	5-5-81	23	3	0	2	16	90	15
100	3-10-81	74	20	0	23	15	70	2
	3-24-81	87	25	0	17	16	53	2
105	4-15-80	259	145	0	8	8	14	0

[a]Only hatches with 20 or more chickens are summarized.

[b]Excess males were culled. Females were normally culled only when afflicted with lameness or other problems.

[c]Mortality (%) = [(number dead)/(number hatched - number culled)] x 100

[d]Incidence of MD (%) = [(number of cases of MD reported)/(number hatched - number culled)] x 100

Table 21. Bursal Weights (Grams) by B Genotype and Sex for 16-Week-Old (6-1 x 15-1) F2 Chickens MD-Vaccinated at Hatching

	B Genotype:		
	2/2	2/5	5/5
Males	1.80	0.26	1.11[b]
	1.18	0.16[b]	0.44
	0.38		0.21
	0.33		
Females	1.78	2.39	0.79
	1.66	1.63	0.75
		1.28	0.28
	$\bar{x}$ = 1.19[a]	$\bar{x}$ = 1.14	$\bar{x}$ = 0.60

[a]mean of both sexes

[b]visceral tumors present

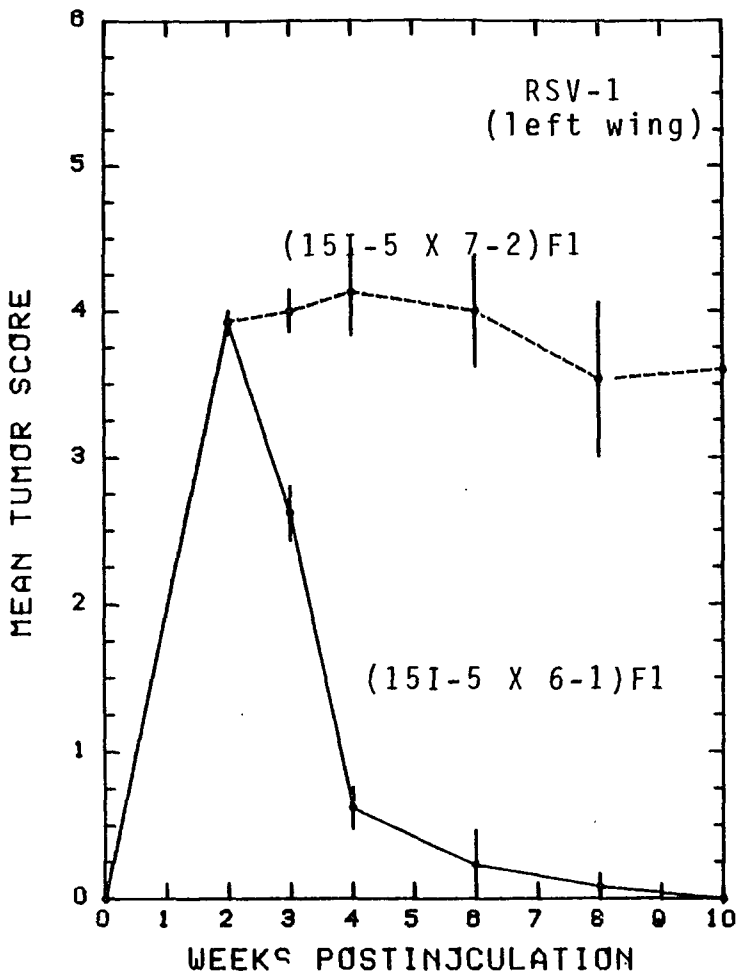

Figure 14A

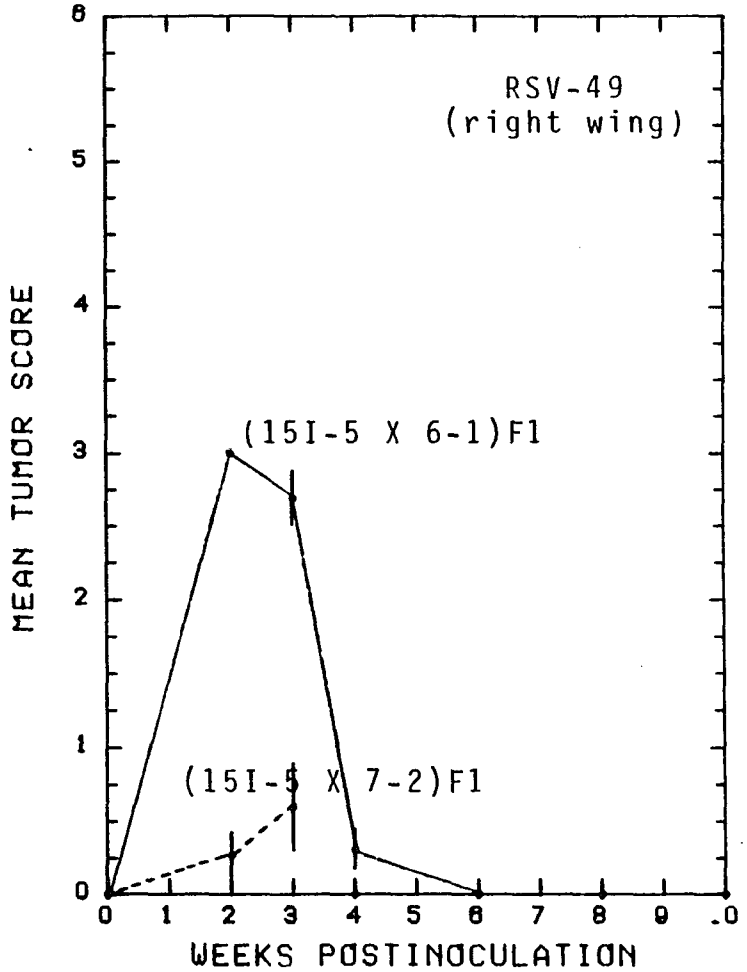

Figure 14B

Figures 14A and B.
Mean left (Fig. 14A) and right (Fig. 14B) wing tumor scores by weeks post-inoculation for (15I-5 X 6-1)F1 and (15I-5 X 7-2)F1 chickens (solid and dashed lines, respectively) challenged in the left wingweb with RSV-1 and in the right with RSV-49. Chickens were vaccinated for Marek's disease prior to hatching (18th day of incubation). Standard errors are represented by vertical lines.

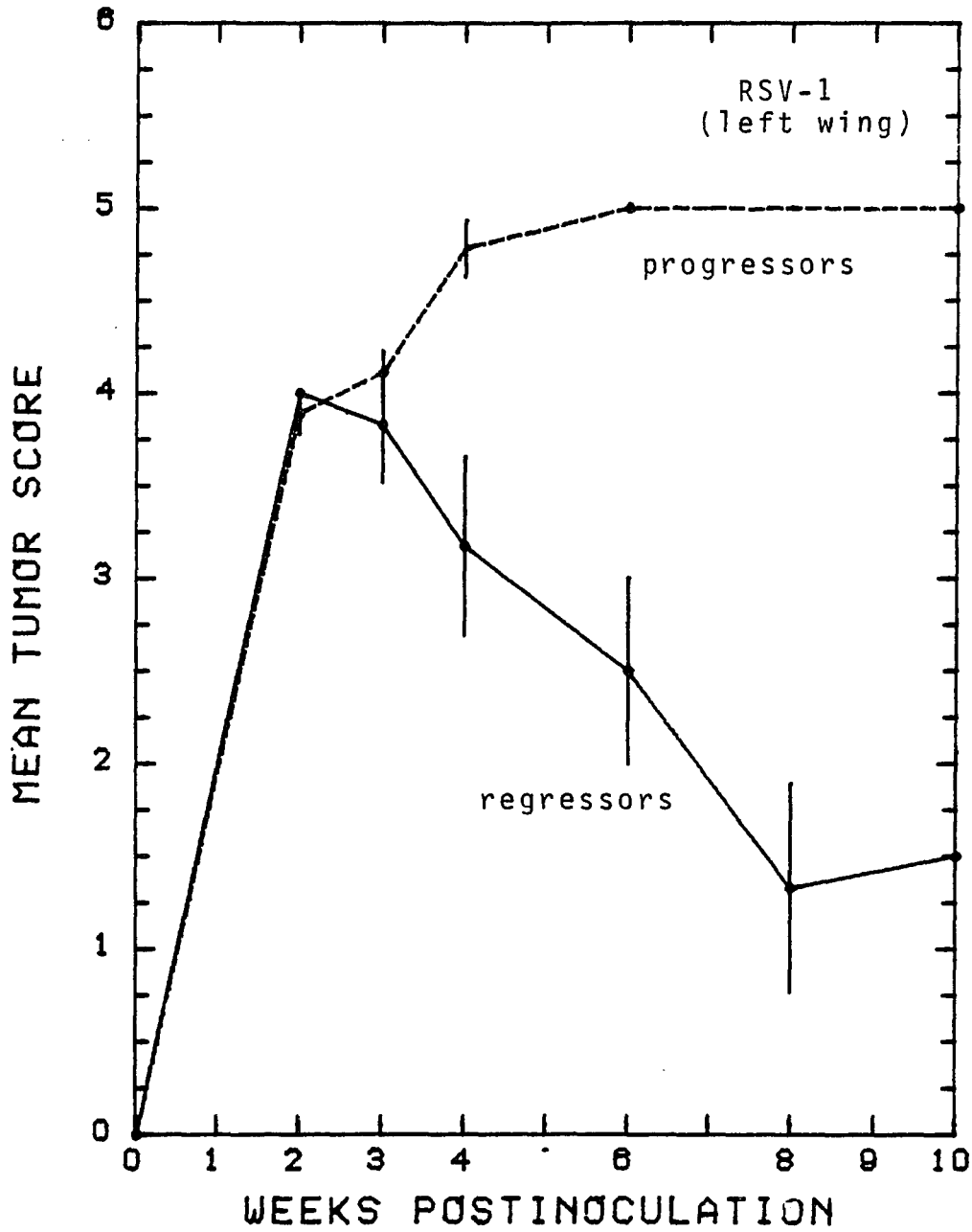

Figure 15.
Mean left wing (RSV-1 induced) tumor scores by weeks postinoculation for (15I-5 X 7-2)F1 chickens (six regressors and nine progressors - solid and dashed lines, respectively). Chickens were MD-vaccinated prior to hatching (18th day of incubation). Standard errors are represented by vertical lines.

growth than observed previously. Figure 15 (page 104) compares mean RSV-1 induced tumor scores of survivor and progressor (15I-5 X 7-2)F1's. Variation in anti-tumor response cannot be explained on the basis of dosage since tumor size at two weeks PI is similar for both regressors and progressors. Regression observed among (15I-5 X 7-2)F1's indicates that such chickens are capable of adequate anti-sarcoma response under suitable environmental circumstances.

Virus Subgroup

Both group and subgroup specific determinants have been shown to be involved in anti-Rous sarcoma response (Ignjatovic *et al*. 1978, Hall *et al*. 1979). In order to determine whether or not the response of a given B genotype to RSV-induced tumors was specific for virus subgroup, each of four different F2 populations were challenged with three subgroups of RSV (RSV-1, RSV-2, and RSV-49 of subgroups A, B, and C, respectively).

In the three F2 populations in which the B2 haplotype occurred, B2 response against RSV-1 and RSV-49 induced tumors was high, but response against RSV-2 induced sarcomas was intermediate. Lower response to RSV-2 induced sarcomas was not due to virus overdosage, as judged by tumor size at two weeks PI. The rapid regression of RSV-1 and RSV-49 induced tumors must therefore have been due in part to high response against subgroup specific antigenic determinants.

The B15 haplotype responses to RSV-1 and RSV-2 induced sarcomas were relatively low, while response to RSV-49 was characterized by a fairly high incidence of regression, again suggesting subgroup specificity for anti-Rous sarcoma response.

Three B haplotypes (B5, B23, and B24) were characterized by low responses against tumors induced by each of the three virus subgroups. Such low responses could indicate inability of these haplotypes to respond to both group and subgroup specific antigenic determinants. Alternatively, the responses associated with these B haplotypes may be suppressed due to exposure to an environmental variable such as Marek's disease. The characteristic MD susceptibilities of the B23 and B24 haplotypes have not been reported. The B5 haplotype, however, has been shown to be susceptible to MD. Among (6-3 X 15-1)F5 chickens challenged at two weeks of age with the JM strain of MD virus, 60% of B5/B5, 22% of B2/B5, and 13% of B2/B2 birds developed MD (Bacon *et al.* 1981). Therefore, if MD exposure is indeed the cause of suppressed anti-tumor response observed among (15I-5 X 7-2)F1 chickens (see discussion concerning possible influence of MD, page 97), then MD-susceptible B haplotypes such as B5 would be subject to the same immunosuppression.

Stress

The social interactions which occur among chickens in

the formation of dominance hierarchies may result in stress in spite of attempts to minimize competition for food and water. Since physiological results of stress can manifest themselves in the compromise of the immune response (Monjan and Collector 1977), it is conceivable that stress could modify anti-Rous sarcoma response.

Under the assumption that increased levels of corticosteroids resulting from stress may result in decreased anti-Rous sarcoma response, B2/B2 and B15/B15 (15I-5 X 6-3)F2 chickens were given intramuscular injections of dexamethasone prior to and following challenge with RSV-1. Anti-tumor response did not appear to be compromised by treatment with the corticosteroid.

The uniform anti-tumor responses observed among (15I-5 X 6-1)F1 chickens following RSV challenge perhaps provided stronger evidence that stress was not an important factor in anti-Rous sarcoma response. Had stress been an important source of variation, then a wider spectrum of responses should have been observed. The greater amount of within B genotype variation in anti-tumor response observed among F2 generation chickens and chickens from noninbred lines could therefore best be explained by genetic variation and perhaps genetic by environmental interaction.

The observed variation in anti-sarcoma response in previous studies with inbred lines could reflect ALV contamination, exposure to MD virus, or other possible environmental variables. Inbred lines, on the other hand,

could conceivably be less capable of adjusting to stressful conditions and therefore more subject to the resultant immunosuppressive effects.

Interaction of MHC Haplotypes in Relation to Rous Sarcoma Regression

RSV-transformed avian cells have been shown to express a variety of antigens relevant to the immune response (Ignjatovic et al. 1978, Hall et al. 1979). Therefore, response against tumor cells could involve interactions with a wide spectrum of antigenic determinants, each contributing in varying degree to the observed anti-tumor response. A B haplotype could be characterized by high responses to some determinants and low responses to others. Two B haplotypes, each with a different characteristic spectrum of responses, could conceivably complement each other if some high responses were dominant in nature allowing such responses to sum across the two haplotypes. Alternatively, a hybrid molecule produced by heterozygotes could provide responses absent or greatly reduced in both parental B types. Complementation for anti-Rous sarcoma response involving both the MHC and non-MHC genes has been reported (Cutting et al. 1981).

Six B genotypes (three B haplotypes) of noninbred line UNH 105 were characterized for response to RSV-induced sarcomas. The B23/B26 genotype had the highest anti-tumor

response, suggesting complementation of the B23 and B26 haplotypes. The matings used to produce the six B genotypes included some producing only one B genotype. Therefore non-MHC influences could account in part for the distribution of responses observed.

Heterozygotes carrying the B24 haplotype appeared to have a lower anti-tumor response than homozygotes for the higher responding B haplotype (either B23 or B26). This could be explained by an Ir gene dose effect similar to that seen with certain hybrid mice in their response to the synthetic terpolymer GLϕ (Dorf et al. 1979). A B23/B23 homozygote, having two doses of the higher responding B23 haplotype, would thus have a greater response than a B23/B24 heterozygote carrying only one dose. An alternative explanation could involve cross-reactivity of B24 alloantigens with tumor antigen(s) responsible for stimulating an anti-tumor response. Tolerance to the B24 alloantigens could result in a decreased response to the cross-reacting tumor antigens and lead to tumor progression. Several studies using various tumor systems in rats and mice have demonstrated such cross-reactivity (Bear et al. 1977, Parmiani et al. 1979, Russell et al. 1979, Paciucci et al. 1980). Heinzelmann et al. (1981b), however, using normal B24/B24 CEF from line 105 and lymphocytes from Rous sarcoma bearing chickens in a cytotoxic assay found no evidence of cross-reactivity.

Attempts to Localize the Non-MHC Genetic Influence on Rous Sarcoma Regression

Endogenous Virus, RAV-0

It was hypothesized that expression of the nondefective endogenous virus, RAV-0, could result in formation of a cell transformation-independent, nonvirion, virus group-specific antigen(s) similar to that described by Wainberg *et al.* (1979a) and that tolerance to such antigen could result in decreased ability to respond to tumors induced by avian leukosis or sarcoma viruses. The high incidence of tumor progression associated with lines 100 and 7-2, RAV-0 producing lines homozygous for the B2 haplotype, may be explained on the basis of this hypothesis. The observation that lines 6-1 and 6-3 have high responses to RSV-induced sarcomas in spite of homozygosity for the defective chf-producing endogenous virus locus, *ev-3*, would also be compatible with this theory.

Experimental results did not support the hypothesis. (15I-5 X 7-2)F1 chickens (hemizygous for *ev-2*, the locus coding for RAV-0) had a high incidence of tumor progression. In contrast, both (6-1 X 7-2)F1 (Table 14, page 83) and (15I-5 X 6-3)F1 X 7-2 chickens (Fig. 2A and B; page 46) were characterized by a high incidence of tumor regression in spite of presence of *ev-2*. The low anti-tumor response associated with (15I-5 X 7-2)F1's, therefore, does not appear to be a result of RAV-0 expression.

Although the anti-Rous sarcoma response associated with the B2 haplotype does not appear to be greatly influenced by expression of chf or RAV-0 in the host, it should not be concluded that such expression would not be capable of suppressing anti-sarcoma responses associated with other B haplotypes. The B2 haplotype may be characterized by high responses to subgroup specific antigenic determinants and thus only slightly affected by tolerance to group specific antigenic determinants shared by the endogenous viruses. If another B haplotype gave high responses against only group specific determinants, it might be much more affected by tolerance to these antigenic determinants if such tolerance occurred.

An attempt was made to immunize chickens against Rous sarcoma challenge using the endogenous virus, RAV-0. Noninbred line K28 chickens were used because of the efficient replication of endogenous viruses in their cells (Robinson et al. 1980). Other treatment groups included chickens immunized with RAV-1 (an exogenous avian leukosis virus), chickens immunized with chf(+) cells from line 6-1 embryos, and control chickens inoculated with diluent alone. Immunization with RAV-0 did not result in enhanced anti-tumor responses (Fig. 12A and B, page 84). RAV-1 immunization resulted in greater resistance to tumor induction with both RSV-1 and RSV-2 (Table 15, page 85) of subgroups A and B, respectively. Subcutaneous inoculations of chf(+) cells from line 6-1 appeared to result in a

decreased rather than enhanced anti-tumor response.

The immunization results are inconclusive. Different routes of anti-tumor immunization have been reported to have contrasting results (Bauer et al. 1979). Immunization of chickens and quail with lectin column-purified antigens from avian sarcoma virus transformed CEF resulted in tumor immunity if immunized intravenously and enhanced tumor growth if immunized intramuscularly. The choice of intramuscular inoculations of RAV-1 and RAV-0 was based upon procedures of Meyers et al. (1972) who used exogenous ALV to increase resistance to challenge with RSV. The choice of subcutaneous inoculation of line 6-1 cells was based upon the procedures of Parmiani and Invernizzi (1975) who used subcutaneous inoculations of normal kidney and liver tissue to induce resistance to methylcholanthrene-induced sarcomas in mice. Dosage of antigen used for inoculation is also known to influence immune responsiveness, both low and high doses being capable of inducing tolerance (Schwartz 1980). Failure to observe enhanced response in RAV-0 immunized chickens or in chickens immunized with 6-1 embryo tissue could therefore reflect a technical difficulty with the immunization procedures rather than a lack of cross-reactivity with tumor antigens involved in anti-tumor response.

The E Blood-Group Locus

Lines 7-2, 100, and 15I-5, all characterized by a high

incidence of progressive Rous sarcoma growth, are homozygous for the E5 haplotype. Regressor lines 6-1 and 6-3 are homozygous for E7. Since the E locus could possibly be linked to a gene affecting anti-sarcoma response, an attempt was made to evaluate a possible "E locus effect" using (15I-5 X 6-3)F1 X 7-2 chickens. No association between E genotype and anti-Rous sarcoma response was detected. E5/E5 and E5/E7 chickens regressed their RSV-induced sarcomas at similar rates (Fig. 13A and B, page 81).

MHC vs Non-MHC Influences on Metastasis

Metastasis is the invasion and spread of neoplastic cells from a primary site to distant organs (Fidler and Hart 1978). With virus-induced tumors, secondary lesions of proliferating neoplastic cells could conceivably result via several different mechanisms:

1) Emboli of tumor cells which have detached from the primary tumor could circulate to small vascular beds of organs where they become arrested. Subsequent invasion of the wall of the arresting vessel and cell multiplication could result in metastasis (Fidler and Hart 1978).

2) Viremia resulting from productive virus-induced tumors could result in transformation of normal target cells at sites distant to the primary tumor.

3) The arrest of virus-producing tumor cell emboli could result in virus-induced transformation of normal surrounding tissue.

No attempt was made to identify the mechanism by which RSV-induced sarcomas became disseminated to distant organs. "Metastatic" lesions were identified on the basis of

histological appearance. Therefore it is conceivable that some lesions classed as metastatic may have resulted from viremia.

Comparisons of B5/B5 (6-1 X 15-1)F6's, B24/B24 line 105's, and progeny of the reciprocal intercrosses following challenge with RSV-1 revealed a considerably higher frequency of metastasis among B5/B5 (6-1 X 15-1)F6 chickens than found among progeny of the other mating types (66% vs 24% or less). In an F2 population resulting from *inter se* matings of [B5/B5 (6-1 X 15-1)F5 X B24/B24 105]F1's the B5/B5 genotype was not associated with a high incidence of metastasis. Therefore it appeared that a non-MHC influence associated with line UNH 105 could result in increased resistance to metastasis.

Behavior could conceivably influence frequency of metastasis. General manipulation of primary invasive neoplasms can cause the release of a shower of tumor emboli into the bloodstream (Fidler and Hart 1978). A chicken line which is highly excitable may be more susceptible to self-inflicted trauma during attempts to escape capture. Flapping of a tumor-bearing wing would clearly result in release of tumor emboli with the potential for metastasis. Line UNH 105 is a non-inbred line of New Hampshires. Lines 6-1 and 15-1 are White Leghorn lines, and they have exhibited a higher degree of excitability than the heavier New Hampshire line. During the course of experimentation, behavior was not considered as a possible influence on

frequency of metastasis. Therefore no accurate observations were made concerning the relative excitability of the F1 and F2 crosses of the New Hampshire and White Leghorn lines.

Another, perhaps more plausible hypothesis could involve an immunosuppressive agent such as Marek's disease virus. Low response to RSV-induced sarcomas could be specific for the tumor antigens involved. In such progressor birds, although T cell cytotoxic activity may be insufficient to control the rapidly growing tumor, other portions of the immune system, such as macrophages and natural killer (NK) cells, may be able to control proliferation of metastatic lesions. In contrast to the case of a specific immunodeficiency, low anti-sarcoma response could result from a generalized immunosuppression as can occur following exposure to MD virus. Sharma (1981) reported that NK activity in MD-resistant lines increased following exposure to MD virus, whereas NK activity in MD-susceptible lines decreased following such exposure. Chickens with progressive Rous sarcoma growth resulting from immunosuppression by MD virus could therefore be more susceptible to metastasis because of decreased NK activity.

The relative MD susceptibility of line 105 has not been reported, but MD has seldom been found in this line at the University of New Hampshire. Thus it would appear that line 105 is fairly resistant to MD. MD resistance associated with the non-MHC background of line 105 could therefore indirectly influence susceptibility to metastasis by

preventing MD-mediated suppression of NK activity.

Depending upon the mechanism of tumor dissemination, properties of the host cell itself could influence such spread. Relative cell susceptibility to virus infection, for example, would clearly influence virus dependent dissemination.

Chemically induced neoplasms have been found to be predominantly heterogeneous, each containing a variety of subpopulations of cells with differing metastatic potentials (Fidler 1978). RSV-transformed chicken fibroblasts, although sharing many common properties related to infection with RSV, can also exhibit heterogeneity. Some RSV-transformed avian cells, for example, are devoid of infectious virus (Freire and Duran-Reynals 1953, Rubin 1962, Wainberg et al. 1979b). Virus devoid tumors are generally found under conditions of tumor regression, metastasis occurring only rarely. Progressive metastatic tumors, in contrast, are characterized by efficient virus production. Although the anti-tumor response of the host appears to have a major effect on such tumor cell heterogeneity, it is conceivable that the properties of the host cell prior to infection could influence the spectrum of cell types obtained following transformation.

VI. SUMMARY AND CONCLUSIONS

Both non-MHC genetic and environmental factors were shown to be important sources of variation in anti-Rous sarcoma response. Avian leukosis virus was found to be a cause of both resistance to tumor induction with RSV-1 and increased susceptibility to progressive tumor growth. Exposure to Marek's disease virus was associated with a hatch effect on anti-tumor response. An experimental hatch with known exposure to MD had generally lower anti-sarcoma responses than subsequent hatches of the same genetic stock.

Using various crosses for evaluation of anti-sarcoma response, it was clearly demonstrated that low responses previously associated with lines 100 and 7-2 were the result of non-MHC genetic influences. In all cases, low anti-Rous sarcoma response was associated with genetic susceptibility to Marek's disease. Histological examination of bursas from chickens that had died as a result of progressive sarcoma growth revealed lesions characteristic of MD. Therefore low anti-sarcoma responses associated with MD susceptible chickens may have been the result of immunosuppression following exposure to MD virus.

Preliminary evidence of a non-MHC influence on susceptibility to metastasis following RSV challenge was obtained. The identity of the non-MHC influence(s) was not determined. However, decreased natural killer cell activity

in MD-susceptible chickens exposed to MD virus would be a possible explanation of the high incidence of metastasis observed in such chickens.

An evaluation of the specificity of anti-Rous sarcoma response revealed that the B2 haplotype had higher responses against RSV-1 and RSV-49 induced sarcomas (subgroups A and C, respectively) than against RSV-2 induced tumors (subgroup B). Such subgroup specificity of anti-Rous sarcoma response had been previously observed by McBride et al. (1981). Some B haplotypes were characterized by low responses to all three subgroups of virus induced tumors. It is plausible that low responses associated with these B haplotypes may have resulted from genetic susceptibility and environmental exposure to MD.

Bacon et al. (1981) suggested that the high susceptibility of the B5 haplotype to ALV-, MD-, and RSV-induced tumors may be the result of a general deficiency in ability to control neoplastic growth. Nordskog et al. (1977) attributed low and high susceptibility to diseases in general to a "fitness" gene linked to the MHC. The results presented in this thesis would suggest that any conclusions concerning the existence of genes affecting general response to neoplasms or to diseases in general should await further experimentation under conditions in which exposure to immunosuppressive agents such as MD virus is strictly controlled. Under conditions of MD exposure, selection for "general fitness" may in reality be selection

for specific resistance to MD. Genotypes with the potential for high resistance to other diseases could be lost in the selection process.

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Project assistantships, 1970-82, University of New Hampshire, N.I.H. funded

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Veterinary practice, small animal, 1977-78, Dover, N.H.

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Brown, D.W., Collins, W.M., Ward, P.H., and Briles, W.E. Complementation of major histocompatibility haplotypes in regression of RSV-induced tumors in noninbred chickens. Poultry Sci 61:409-413, 1982.

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