

A GENETIC STUDY OF THE MURINE HUMORAL
IMMUNE RESPONSE TO MCF-247 VIRUS

Submitted in partial fulfillment of the requirements
for graduation with honors to the Department of Biology
at Carroll College, Helena, Montana

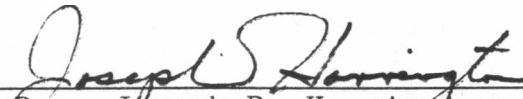
Melchisedek L. Margaris

March 22, 1983

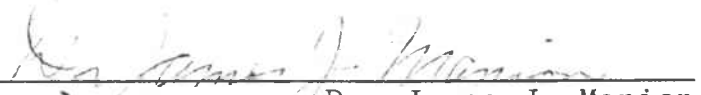
CORETTE LIBRARY
CARROLL COLLEGE



This thesis for honors recognition has been approved
for the Department of Biology.



Rev. Joseph D. Harrington, Advisor



Dr. James J. Manion



Mr. Guido Bugni

March 22, 1983

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Miles (Todd) Cloyd of Rocky Mountain Laboratories, whose guidance, explanations, encouragement, and patience made this thesis possible. I wish to thank Margie Thompson, Kathy Wehrly, Jane Nishio, Dr. Bill Britt, Frank McAtee, and Frank Malek, who taught me laboratory techniques and aided me in times of confusion. I am also grateful to Rocky Mountain Laboratories for allowing me the use of their facilities, equipment, and supplies. I extend thanks to Father Harrington, my advisor, and my readers, Mr. Guido Bugni and Dr. James Manion, all of whom have encouraged as well as educated me during my years at Carroll. I thank Cheryl McNurlin for skillfully handling the task of typing this thesis. Last, but not least, I wish to thank my wonderful family, whose love and support gave me the strength to go on.

ABSTRACT

Mink cell focus-inducing virus MCF-247 was inoculated into various strains of mice to determine whether any of these strains mount a humoral immune response to the virus. Finding that there were both responders and nonresponders, a genetic study was conducted in order to determine what controls the immune response. C57BL/6J mice (high antibody responders) and DBA/2J mice (nonresponders) were crossed and the genetics of their F₁, F₂ and Backcross to DBA/2J mice were followed. Indirect immunofluorescent assays determined that the F₁ generation uniformly produced a high antibody titer when inoculated with the virus. The F₂ and Backcross generations were found to have high responders (titer \geq 80), low responders (titer $<$ 80), and nonresponders (titer \leq 10) to the inoculated virus. All mice were inoculated as babies and were tested 1-6 months after injection. Various genotypic models were proposed for the genetic control of the immune response. It appears that at least one and possibly two genes are involved in control of the immune response.

Possible association of the mouse H-2 major histocompatibility complex, shown to regulate various immune

responses (12), with the immune response to inoculated MCF-247 virus was investigated. Hemagglutination tests indicated that there does not appear to be any association between the two. Rather, the immune response appears to be like that to type III pneumococcal polysaccharide, in which nonH-2 genes control the immune response (13).

Possible links between the immune response gene(s) and the coat color gene b were investigated for the purpose of aiding in the mapping of the immune response gene(s). It was determined that the two are not linked. The immune response was also determined to be a nonsex-linked trait. Further research of the immune response is indicated.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..... ii

ABSTRACT.....iii

LIST OF TABLES..... vi

INTRODUCTION AND LITERATURE REVIEW..... 1

MATERIALS AND METHODS..... 6

OBSERVATIONS AND RESULTS..... 11

 Immunofluorescence Assays..... 11

 Lack of Association of the H-2 Complex with
 Ab Response..... 14

 Non-linkage of the Coat Color Gene b with
 Ab Response..... 15

DISCUSSION AND CONCLUSION..... 49

LITERATURE CITED..... 54

LIST OF TABLES

Table

1	Mouse Strain Survey of Antibody Response to Inoculated MCF-247 Virus.....	17
2	Antibody Response in C57/BL6J, DBA/2J and Hybrids Inoculated With MCF-247 Virus.....	18
3.1	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross Based on a 1 Gene Immune Response Control Model.....	19
3.2	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Cross Based on a 1 Gene Immune Response Control Model.....	20
4.1	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J)F ₂ Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer \geq 20	21
4.2	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J)F ₂ Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer \geq 40	22
4.3	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J)F ₂ Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer \geq 80	23

Table

4.4	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 20	24
4.5	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 40	25
4.6	Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 80	26
5.1	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross Based on a 2 Gene Immune Response Control Model.....	27
5.2	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Cross Based on a 2 Gene Immune Response Control Model.....	28
6.1	Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F ₂ Mice, With the Requirement for a High Positive Antibody Response being a Titer ≥ 80 and for a Low Positive Antibody Response Being < 80 and ≥ 20	29

Table

6.2 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being < 80 and ≥ 4030

6.3 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 20 31

6.4 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 40 32

6.5 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 80 33

6.6 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being a Titer < 80 and ≥ 20 34

Table

6.7 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being a Titer < 80 and ≥ 4035

6.8 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 20 36

6.9 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 4037

6.10 Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 8038

7.1 Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios for H-2 Haplotype Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross.....39

7.2 Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios for H-2 Haplotype Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Backcross.....40

Table

7.3	Observed H-2 Haplotypes of the (C57BL/6J X DBA/2J)F ₁ Generation Exhibiting a Positive Antibody Response (Titer \geq 20) to Inoculated MCF-247 Virus.....	41
7.4	Observed H-2 Haplotypes of the (C57BL/6J X DBA/2J)F ₂ Generation and the (C57BL/6J X DBA/2J) X DBA/2J Backcross Generation Exhibiting a Positive Antibody Response (Titer \geq 20) to Inoculated MCF-247 Virus.....	41
7.5	Chi Square Analysis of the Association of the H-2 Major Histocompatibility Complex with the Humoral Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F ₂ Generation.....	42
7.6	Chi Square Analysis of the Association of the H-2 Major Histocompatibility Complex with the Humoral Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Generation.....	43
8.1	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios for Coat Color Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross.....	44
8.2	Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios for Coat Color Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Backcross.....	45
8.3	Coat Colors of (C57BL/6J X DBA/2J) F ₂ Mice and (C57BL/6J X DBA/2J) Backcross Mice Showing a Positive Antibody Response (Titer \geq 20) to Inoculated MCF-247 Virus.....	46
8.4	Chi Square Analysis of the Association of the Coat Color Gene <u>b</u> with the Humoral Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F ₂ Generation.....	47

Table

8.5 Chi Square Analysis of the Association
of the Coat Color Gene b with the Humoral
Immune Response to Inoculated MCF-247
Virus in the (C57BL/6J X DBA/2J) X
DBA/2J Backcross Generation48

INTRODUCTION AND LITERATURE REVIEW

RNA tumor viruses are very common in nature. They are capable of causing leukemias and sarcomas in most animals. They are the only known viruses which can become "part of the animal." Some of these viruses may actually become incorporated into the animal's genome and be passed on as part of the genetic content of the gametes (1).

The etiological role of virus in murine leukemia was first suggested by the now classical studies conducted by Gross, the results of which were published in 1951. These studies demonstrated that cell-free preparations of lymphoid tissue from leukemic mice were leukemogenic when inoculated back into newborn mice. In 1970, Gross reported success in isolation of the virus and induction of leukemia with purified virus. This confirmed the viral etiology of the particular murine leukemia virus under study at the time. Other investigations throughout the 1970's led to the belief that a viral genome is incorporated into the cellular genome of all mouse cells, is inherited through the germ line, and behaves essentially as a host gene under the same regulatory influences as other cellular genes (2). It is now believed that

at least 0.01% of the mouse genome is composed of viral genomes (proviruses) (3).

The murine leukemia viruses (MuLVs) endogenous to laboratory mice have been divided into three related classes on the basis of their host range in tissue culture, which is determined by the viral envelope glycoprotein, gp70, encoded by the viral gene env. The MuLVs which infect only murine cells are placed in the ecotropic virus class. Those that replicate primarily in heterologous cells are placed in the xenotropic virus class. The third class includes the mink cytopathic focus-forming (MCF) viruses, which can grow in both murine and heterologous cells. Various studies have suggested that MCF viruses are genetic recombinants between ecotropic MuLV and endogenous env sequences related to xenotropic viruses and some of these viruses have been implicated in causing leukemia in mice (4,5). For this reason, the MCF viruses have been the subject of much study in recent years.

MCF viruses are designated as such because of their ability to induce focal growth or morphological alterations in monolayers of the mink lung cell line ATCC no. CCL-64 (6,7,8). This is the type of assay used to quantitate MCF virus infectivity (6,8). The original isolate of MCF virus was obtained from a thymic extract of a 6-month old AKR mouse. The results of this study were reported in 1977 (8). This gave rise to the prototype strain no. 247 (8). Since that time, considerable

effort has been expended in the characterization of various MCF viruses. The data obtained so far indicates that MCF viruses act as the proximal carcinogens in the induction of a variety of hematopoietic neoplasms (6). However, the exact origin and role of these viruses in tumorigenesis is still incompletely understood (4). Various studies (3,8) have found that the MCF viruses have the following unique properties:

- (1) They are able to infect both murine and heterologous cells. However, they do not infect as many species as xenotropic viruses do (M.W. Cloyd, personal communication).
- (2) MCFs demonstrate envelope properties of both xenotropic and ecotropic viruses.
- (3) In addition to expressing both ecotropic and xenotropic-like antigens, they also express "MCF specific" antigens not found on the surface of cells infected with ecotropic or xenotropic viruses.
- (4) Unlike other viruses of the mouse, MCFs can only be isolated from leukemic or preleukemic cells. In mice with high amounts of ecotropic virus expression, MCFs are found in close temporal association with the development of leukemia. It was thus inferred from this that MCFs were associated with leukemia.
- (5) Certain MCF viruses have been shown to induce leukemia when inoculated back into mice but this effect

is mouse strain specific; i.e., only certain strains are susceptible to leukemia induction by MCF virus.

- (6) They have a distinct cytopathic effect on mink lung cells.

Since an immune response may play an important role in preventing induction of leukemia by MCF virus, this study was initiated to examine the humoral immune response in mice to inoculated MCF-247 virus. The following objectives were in mind: (1) To determine which, if any, mouse strains mounted an immune response to the MCF-247 virus, and (2) To determine what controls this response, if it is present. Very little previous work has been done in this area (9,10,11).

Preliminary studies indicated that most mouse strains mount an immune response to MCF-247, whereas only a few strains do not (notably the AKR and DBA/2 strains; see table 1). We chose to examine the humoral immune response in the C57BL/6 and DBA/2 strains combinations because these mice differ at many genetic loci which can be followed to map newly discovered genes. Since the H-2 major histocompatibility complex has been shown to regulate many immune responses in the mouse, it was also studied in order to determine if it played any role in the immune response to MCF-247 (or the lack of one) in these mice. An indirect immunofluoroassay was employed to monitor the presence of serum antibody to MCF-247 and a hemagglutination test was employed

in the study of the role of H-2 in the response. Coat color of responders and nonresponders was also followed in order to determine if the immune response was linked to the coat color gene b.

MATERIALS AND METHODS

Mice

C57BL/6J and DBA/2J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. These mice and their hybrids were bred at the Rocky Mountain Laboratory, Hamilton, Montana. Test mice were inoculated as sucklings (2-5 days of age) or as weanlings (approx. 1 mo of age) with 0.04-0.05 ml of MCF-247 virus suspension (approx. $10^{2.8}$ - $10^{3.5}$ focus forming units). Injection was done intraperitoneally.

Serological Assays and Indirect Immunofluorescence Assay

Antibody response to the injected MCF-247 virus was assayed by an indirect immunofluorescence test. Mice were bled from the tail 1-6 months after infection, using 300ul heparinized capillary tubes (Caraway). The capillary tubes were spun in a micro-hematocrit centrifuge for 5 minutes and the serum transferred to plastic vials which were then kept on ice or frozen (-70°C , Harris Freezer) until needed.

SC-1 cells (an embryo cell line from a feral mouse, Hartley and Rowe, 1975) infected with MCF-247 virus (hereafter referred to as SC-1-247 cells) were used to test for the presence of antibody, with uninfected

SC-1 cells serving as controls.

For each assay, a micro-titer plate (Disposable Polystyrene 96 u-well micro-titration plates, Titertek) was prepared by wetting the individual wells with 95% ethyl alcohol, which enhances the adherence of cells to the walls of the wells, and then allowing the plate to dry. Experimental and control assays were done for each serum sample.

For the experimental assay, doubling dilutions of serum from test mice were prepared, beginning at 1:10 and ending at 1:160. The 1:10 dilution was prepared by adding 90ul of phosphate buffered saline to the wells of the first column (well #1) and then adding 10ul of serum from test mice to their respective well. 50ul of phosphate-buffered saline was added to each well in columns 2-5. 50ul of the mixture in well #1 was then transferred to well #2 and mixed, and so on, effecting a doubling serum dilution. 50ul was removed from the last well after transferring and mixing and discarded. Serum dilutions for the control wells went from 1:10 to 1:40, using columns 7, 8, and 9, and were prepared as above.

SC-1-247 and uninfected SC-1 cells were grown in tissue culture. They were removed from the flasks by trypsinization, counted using a hemocytometer, centrifuged, and resuspended in Dulbecco's Medium at a concentration of 1×10^7 cells per ml. 10ul of the SC-1-247

suspension was pipetted into each experimental well. 10ul of uninfected SC-1 cells was pipetted into each control well.

The wells were covered with pre-cut acetate sealing tape (Dynatech Laboratories, Inc.) and the tray was shaken to suspend the cells. The plate was then incubated at room temperature for 30 min, with occasional shaking. After incubation, the plate was centrifuged at 1200rpm (200xg) for 2 min. The supernatant fluid from each well was removed via aspiration. One drop of Dulbecco's Medium was added to each well, and the cells were resuspended by shaking. The plate was then recentrifuged at 1200rpm (200xg) for 2 min. The supernatant was removed from each well via aspiration. After this washing procedure, the cells were resuspended in fluorescein-conjugated goat anti-mouse Immunoglobulin G, the optimum dilution of which was determined by previous titration. Incubation and washings were the same as with the mouse sera. The final cell pellets were each suspended in one drop of a solution that was 50% glass distilled saline, 50% glycerin, and 2.5% formalin, and mounted under coverslips on microscope slides.

Cells were examined using a Leitz Orthoplan incident-light fluorescence microscope with ultra-violet illumination, at 400x. Cells were scored for membrane fluorescence, which indicated the presence of an antigen-antibody complex on the surface of the cell. This was an indirect

indication that antibodies were present in the serum and bound to cell surface antigens. The highest dilution of serum that resulted in fluorescing cells was determined, which indicated the relative quantity of antibody present.

The hemagglutination technique used for H-2 typing was as follows: An 1.8% dextran solution was prepared by mixing .36ml stock (10%) dextran (ave. m.w. 110,000-115,000, obtained from Glaxo Laboratories Ltd, Greenford, England) with 1.64ml of glass distilled saline. The anti-H2^b antibody used was IM-3, an antiserum from (BALB/c x A)F₁ mice immunized with C57BL/10 cells. The anti-H2^d antibody used was 25-42, an antiserum obtained from C57BL/10 mice immunized with B10.D2 cells. Doubling serial dilutions beginning at 1:40 and ending at 1:320 were prepared with each antiserum, the dextran solution being used as the diluting agent.

For each mouse tested, 25ul of the above diluted antibody suspensions were placed in the appropriate wells of a 96u-well micro-titer plate (Disposable Polystyrene 96u-well micro-titration plates, Titertek). Control wells containing 25ul of the dextran-saline solution without antiserum were also prepared for each mouse.

Mice were bled from the tail and 1 drop of blood was collected in 10ml of saline. 3ml of this suspension was centrifuged, the supernatant aspirated, and the

RBC's resuspended in 1ml of 50% fetal calf serum (FCS) in saline. 25ul of this suspension was pipetted into each well. The plate was covered with pre-cut acetate sealing tape (Dynatech Laboratories, Inc.) and then shaken to suspend the cells. The plate was then refrigerated overnight. Hemagglutination was scored the following day. A positive result was recorded when the RBC's remained in a lattice coating the walls of the microtiter well. A negative result was recorded when the RBC's formed a small button of packed cells on the bottom of the well.

OBSERVATIONS AND RESULTS

The observations and results have been divided into three sections. The first section, Immunofluorescence Assays, presents the data obtained from indirect immunofluorescence assays (IFAs) for antibody production in mice inoculated with MCF-247 virus and the results of Chi Square analysis of the validity of various proposed gene models for the control of the immune response in (C57BL/6J X DBA/2J) hybrid mice, using IFA data. The second section, Lack of Association of the H-2 Complex with Ab Response, presents the data obtained from H-2 typing tests on antibody producing and nonantibody producing mice. Chi Square analysis of this data showing that there is no association between the H-2 major histocompatibility complex and the humoral immune response to MCF-247 virus is also presented. The third section, Non-linkage of the Coat Color Gene b with Ab Response, presents data and results of Chi Square analysis indicating that the coat color gene b does not appear to be linked to the humoral immune response to inoculated MCF-247 virus in (C57BL/6J X DBA/2J) hybrid mice.

Immunofluorescence Assays

Seven different mouse strains were tested for anti-

body response to inoculated MCF-247 virus (Table 1). Only two (AKR and DBA/2) did not mount a response; the others did. The results of the strain survey led to the selection of the C57BL/6J strain and the DBA/2J strain for the genetic study. By studying the F_1 , F_2 , and Backcross generations, we could follow segregation of the response phenotype and compare the results to expected phenotypes for a 1 gene immune response control model.

The strains and their hybrids were tested for antibody response to inoculated MCF-247 virus. The results of the immunofluorescence assays (IFAs) are presented in Table 2. It was found that the response was dominant since the F_1 generation uniformly possessed high amounts of antibody. The backcross was then made to the nonresponsive parent, DBA/2J. 41-61% of the inoculated backcross mice and 56-75% of the inoculated F_2 mice made an antibody response, depending on what antibody titer is considered a positive response. Tables 3.1 - 3.2 show the potential gametes generated and the expected genotypic and phenotypic ratios for both the Backcross to DBA/2J and the F_2 generations, based on a 1 gene immune response control model.

The Chi Square test was used to determine the validity of a 1 gene immune response control model in the F_2 and backcross generations using data from the immunofluorescence assay. In order to examine all possibili-

ties, a range of titer values were used as positive response cutoff points (1/20, 1/40, 1/80). The results of the statistical analyses are presented in Tables 4.1 - 4.6.

The results lend support to a 1 gene model (Tables 4.1 - 4.2, 4.4 - 4.6). A titer of 20 or greater was chosen as the cutoff point for a positive response for later tests (H-2 and coat color association), in part because of the Chi Square analyses and in part because no DBA/2J mouse tested produced an antibody titer > 10 .

The possibility of two genes being involved in the humoral immune response in the mice was also considered. Two different models were formulated: (1) A 2 gene control model in which inheritance of either gene is sufficient to bring about an immune response, which would be classified as a high response if both dominant alleles were inherited, and classified as a low response if only one dominant allele was inherited; (2) A 2 gene control model in which inheritance of two dominant alleles is required for an immune response. The validity of the various models was evaluated by the Chi Square Test. The results are contained in Tables 6.1-6.10. Tables 5.1 - 5.2 show the potential gametes generated and the expected genotypic and phenotypic ratios for the Backcross and the F_2 generations for each 2 gene model.

Although the results of the Chi Square analyses

seem to rule out the possibility of a 2 gene model in which inheritance of either gene is sufficient to bring about an antibody response, (Tables 6.1 - 6.3, 6.6 - 6.10), there is some suggestion (see Tables 6.4 and 6.5) that a 2 gene model requiring the inheritance of two dominant genes for an antibody response may be applicable.

Lack of Association of the H-2 Complex with Ab Response

The H-2 Major Histocompatibility Complex of the mouse may be defined serologically (12). The haplotype for the C57BL/6J strain (the positive antibody responder) is defined as H-2^b and that for the DBA/2J strain (the negative antibody responder) is defined as H-2^d. The potential gametes for this trait and the expected genotypic and phenotypic ratios for the F₂ and Backcross hybrids were determined (Tables 7.1 - 7.2). The H-2 haplotype of the F₁ generation (all positive antibody responders) can be determined by simple genetics as H-2^{b/d}. This was confirmed serologically via a hemagglutination technique (Table 7.3). The haplotypes of the F₂ and Backcross generations were also followed serologically via the same hemagglutination technique (Table 7.4).

Neither visual inspection of the raw data or Chi Square analysis of it suggest association of the H-2 Complex with the humoral immune response to MCF-247 virus in the F₂ and Backcross hybrids (Tables 7.5, 7.6).

Non-linkage of the Coat Color Gene *b* with Ab Response

Because linkage of coat color genes with those of the humoral immune response to MCF-247 virus would provide information on the location of the immune response gene(s) for mapping purposes, the gene which determines whether coat color is black or brown was followed in the immune response study. This coat color gene is located on Chromosome #4 in the mouse and is designated as the *b* locus. A mouse with the *bb* haplotype will have brown hair. This is the case for the DBA/2 strain. A mouse with the *bb*⁺ or *b*⁺*b*⁺ haplotype will have black hair. The C57BL/6 strain has the *b*⁺*b*⁺ haplotype. The F₁ generation resulting from a cross between these two strains has the *b*⁺*b* haplotype, and hence, the black coat color. The *b*⁺ allele is the wild type and is dominant. From the data in Table 1, it would be expected that if the coat color gene is linked to the humoral immune response, all positive responders will have at least one *b*⁺ locus.

Potential gametes and expected genotypic and phenotypic ratios for this coat color trait were determined for the F₂ and Backcross generations (Tables 8.1, 8.2). The observed coat colors of F₂ and Backcross mice exhibiting a positive antibody response are recorded in Table 8.3. Chi Square Analysis of possible linkage of the coat color gene *b* with the humoral immune response gene(s) for the F₂ and Backcross hybrids suggests no linkage

of the coat color gene with any gene controlling the humoral immune response to MCF-247 virus (Tables 8.4, 8.5).

Table 1

Mouse Strain Survey of Antibody Response
to Inoculated MCF-247 Virus*

<u>Mouse Strain</u>	<u>Number of Mice Tested</u>	<u>Approximate antibody titer to MCF-247 virus</u>
SWR/J	3	1:80 - 1:320
SJL/J	4	1:80 - 1:160
AKR/J	6	1:10 or less
NFS/N	6	1:160 - 1:320
DBA/2J	6	1:10 or less
DBA/1J	4	1:80 - 1:160
C57BL/6J	6	1:80 - 1:320

*All mice were inoculated as babies and tested as adults (2-6 mo old).

Table 2

Antibody Response in C57BL/6J, DBA/2J and Hybrids
Inoculated With MCF-247 Virus

Strain or Cross	Number of mice with antibody response		
	(Titer \geq 20)	(Titer \geq 40)	(Titer \geq 80)
Parental C57BL/6J	6/6	6/6	6/6
DBA/2J	0/6	0/6	0/6
F ₁ C57BL/6J X DBA/2J	6/6	6/6	6/6
Backcross to DBA/2J (C57BL/6J X DBA/2J) X DBA/2J	27/44	24/44	18/44
F ₂ (C57BL/6J X DBA/2J)F ₂	24/32	22/32	18/32

Table 3.1

Potential Gametes Generated and Expected Genotypic
and Phenotypic Ratios Resulting From a
(C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross
Based on a 1 Gene Immune Response Control Model

Gene alleles (Both parents): A|a
Potential Gametes (Both parents): A,a
Result of cross:

	A	a
A	AA	Aa
a	Aa	aa

Antibody Responder Genotype: AA, Aa

Nonresponder Genotype: aa

Expected Phenotypic Ratios:

3 antibody producers:1 non-responder

Table 3.2

Potential Gametes Generated and Expected Genotypic
and Phenotypic Ratios Resulting From a
(C57BL/6J X DBA/2J) X DBA/2J Cross
Based on a 1 Gene Immune Response Control Model

Gene alleles of Parents:

(C57BL/6J X DBA/2J): A|a
DBA/2J: a|a

Potential Gametes Formed:

(C57BL/6J X DBA/2J): A, a
(DBA/2J): a

Result of Cross:

	a	a
A	Aa	Aa
a	aa	aa

Expected Phenotypic Ratios:
1 antibody responder:1 non-
responder

Table 4.1

Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 20

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	24	8	32
Expected number	24	8	32
(Obs. - Exp.)	0	0	0
(Obs. - Exp.) ²	0	0	
(Obs. - Exp.) ² /Exp.	0	0	0

* $\chi^2 = 0$

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

$$*\chi^2 = \sum [(\text{Obs.} - \text{Exp.})^2 / \text{Exp.}]$$

Table 4.2

Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer \geq 40

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	22	10	32
Expected number	24	8	32
(Obs. - Exp.)	-2	2	0
(Obs. - Exp.) ²	4	4	
(Obs. - Exp.) ² /Exp.	0.1667	0.5	0.6667

X² from calculations = 0.6667

From the Chi Square Table, X²_{0.05,1} = 3.841

Table 4.3

Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) F_2 Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer \geq 80

	Number of Mice		Total
	Ab pos.	Ab neg.	
Observed number	18	14	32
Expected number	24	8	32
(Obs. - Exp.)	-6	6	0
(Obs. - Exp.) ²	36	36	
(Obs. - Exp.) ² /Exp.	1.5	4.5	6

$$\chi^2 = 6$$

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

Table 4.4

Chi Square Analysis of IFA Data for a l Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 20

	Number of Mice		
	<u>Ab pos.</u>	<u>Ab neg.</u>	<u>Total</u>
Observed number	27	17	44
Expected number	22	22	44
(Obs. - Exp.)	5	-5	0
(Obs. - Exp.) ²	25	25	
(Obs. - Exp.) ² /Exp.	1.1364	1.1364	2.2727

$\chi^2 = 2.2727$

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

Table 4.5

Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 40

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	24	20	44
Expected number	22	22	44
(Obs. - Exp.)	2	-2	0
(Obs. - Exp.) ²	4	4	
(Obs. - Exp.) ² /Exp.	0.1818	0.1818	0.3636

$$\chi^2 = 0.3636$$

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

Table 4.6

Chi Square Analysis of IFA Data for a 1 Gene Immune Response Control Model in the (C57BL/6J X DBA/2J) X DBA/2J Hybrid Mice, With the Requirement for a Positive Antibody Response to Inoculated MCF-247 Virus Being a Titer ≥ 80

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	18	26	44
Expected number	22	22	44
(Obs. - Exp.)	-4	4	0
(Obs. - Exp.) ²	16	16	
(Obs. - Exp.) ² /Exp.	0.7273	0.7273	1.4546

$$\chi^2 = 1.4546$$

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

Table 5.1

Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross Based on a 2 Gene Immune Response Control Model

Gene Alleles of Parents: A|aB|b

Potential Gametes Formed (Both Parents): AB, Ab, aB, ab

Punnett Square For Cross:

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

Expected Phenotypic Ratios:

For an immune response requiring only 1 of 2 Dominant Genes: 9 High Responders:6 Low Responders:1 Nonresponder

For an immune response requiring 2 Dominant Genes: 9 Responders:7 Nonresponders

Table 5.2

Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Cross Based on a 2 Gene Immune Response Control Model

Gene Alleles of (C57BL/6J X DBA/2J) Parent (B6D2): A|a B|b

Gene Alleles of DBA/2J (D2) Parent: a|a b|b

Potential Gametes Formed

B6D2 Parent: Ab, Ab, aB, ab

D2 Parent: ab

Punnett Square for Cross:

	ab
AB	AaBb
Ab	Aabb
aB	aaBb
ab	aabb

Expected Phenotypic Ratios:

For an immune response requiring only 1 of 2 Dominant Genes: 1 High Responder:2 Low Responders:1 Nonresponder

For an immune response requiring 2 Dominant Genes: 1 Responder:3 Nonresponders

TABLE 6.1

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes For an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being < 80 and ≥ 20

	Number of Mice			Total
	High Ab rep.	Low Ab rep.	Ab neg.	
Observed number	18	6	8	32
Expected number	18	12	2	32
(Obs. - Exp.)	0	-6	6	
(Obs. - Exp.) ²	0	36	36	
(Obs. - Exp.) ² /Exp.	0	3	18	21

χ^2 from calculations: 21.0

From the Chi Square Table, $\chi^2_{0.05,2} = 5.991$

Table 6.2

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a High Positive Antibody Response Being a Titer \geq 80 and for a Low Positive Antibody Response Being $<$ 80 and \geq 40

	Number of Mice			Total
	High Ab rep.	Low Ab rep.	Ab neg.	
Observed number	18	4	10	32
Expected number	18	12	2	32
(Obs. - Exp.)	0	-8	8	0
(Obs. - Exp.) ²	0	64	64	
(Obs. - Exp.) ² /Exp.	0	5.3333	32	37.3333

χ^2 from calculations: 37.3333

From the Chi Square Table, $\chi^2_{0.05,2} = 5.991$

Table 6.3

Chi Square Analysis of IFA Data For a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer \geq 20

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	24	8	32
Expected number	18	14	32
(Obs. - Exp.)	6	-6	0
(Obs. - Exp.) ²	36	36	
(Obs. - Exp.) ² /Exp.	2	2.5714	4.5714

X² from calculations: 4.5714

From the Chi Square Table, X²_{0.05,1} = 3.841

Table 6.4

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer Level \geq 40

	Number of Mice		
	<u>Ab pos.</u>	<u>Ab neg.</u>	<u>Total</u>
Observed number	22	10	32
Expected number	18	14	32
(Obs. - Exp.)	4	-4	0
(Obs. - Exp.) ²	16	16	
(Obs. - Exp.) ² /Exp.	0.8889	1.1429	2.0318

X² from calculations: 2.0318

From the Chi Square Table, X²_{0.05,1} = 3.841

Table 6.5

Chi Square Analysis of IFA Data For a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Mice, With the Requirement for a Positive Antibody Response Being a Titer Level \geq 80

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	18	14	32
Expected number	18	14	32
(Obs. - Exp.)	0	0	0
(Obs. - Exp.) ²	0	0	
(Obs. - Exp.) ² /Exp.	0	0	0

X² from calculations: 0

From the Chi Square Table, X²_{0.05,1} = 3.841

Table 6.6

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being a Titer < 80 and ≥ 20

	Number of Mice			Total
	High Ab rep.	Low Ab rep.	Ab neg.	
Observed number	18	9	17	44
Expected number	11	22	11	44
(Obs. - Exp.)	7	-13	6	0
(Obs. - Exp.) ²	49	169	36	
(Obs. - Exp.) ² /Exp.	4.4545	7.6818	3.2727	15.4090

X² from calculations: 15.4090

From the Chi Square Table, X²_{0.05,2} = 5.991

Table 6.7

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring Only 1 of 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a High Positive Antibody Response Being a Titer ≥ 80 and for a Low Positive Antibody Response Being a Titer < 80 and ≥ 40

	Number of Mice			Total
	High Ab rep.	Low Ab rep.	Ab neg.	
Observed number	18	6	20	44
Expected number	11	22	11	44
(Obs. - Exp.)	7	-16	9	0
(Obs. - Exp.) ²	49	256	81	
(Obs. - Exp.) ² /Exp.	4.4545	11.6364	7.3636	23.4545

X^2 from calculations: 23.4545

From the Chi Square Table, $X^2_{0.05,2} = 5.991$

Table 6.8

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer \geq 20

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	27	17	44
Expected number	11	33	44
(Obs. - Exp.)	16	-16	0
(Obs. - Exp.) ²	256	256	
(Obs. - Exp.) ² /Exp.	23.2727	7.7576	31.0303

χ^2 from calculations = 31.0303

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

Table 6.9

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer \geq 40

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	24	20	44
Expected number	11	33	44
(Obs. - Exp.)	13	-13	0
(Obs. - Exp.) ²	169	169	
(Obs. - Exp.) ² /Exp.	15.3636	5.1212	20.4848

X^2 from calculations = 20.4848

From the Chi Square Table, $X^2_{0.05,1} = 3.841$

Table 6.10

Chi Square Analysis of IFA Data for a 2 Gene Immune Response Control Model Requiring 2 Dominant Genes for an Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J x DBA/2J) X DBA/2J Backcross Mice, With the Requirement for a Positive Antibody Response Being a Titer ≥ 80

	Number of Mice		
	Ab pos.	Ab neg.	Total
Observed number	18	26	44
Expected number	11	33	44
(Obs. - Exp.)	7	-7	0
(Obs. - Exp.) ²	49	49	
(Obs. - Exp.) ² /Exp.	4.4545	1.4848	5.9393

χ^2 from calculations = 5.9393

From the Chi Square Table, $\chi^2_{0.05,1} = 3.841$

7.1

Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios For H-2 Haplotype Resulting From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross

H-2 Haplotype of Both Parents: b|d

Potential Gametes Formed:
Both Parents: b,d

Punnett Square for Cross:

	b	d
b	bb	bd
d	bd	dd

Proposed genotypes of antibody responders: b|b, b|d

Proposed genotypes of nonresponders: d|d

Expected phenotypic ratio: 3 antibody responders:1 nonresponder

Table 7.2

Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios For H-2 Haplotypes Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Backcross

H-2 Haplotype of (C57BL/6J X DBA/2J) Parent: b|d

H-2 Haplotype of DBA/2J Parent: d|d

Punnett Square for Cross:

	d
b	bd
d	dd

Proposed genotypes of antibody responders: b|d

Proposed genotypes of nonresponders: d|d

Expected phenotypic ratio: 1 antibody responder:1 nonresponder

Table 7.3

Observed H-2 Haplotypes of the (C57BL/6J X DBA/2J)F₁
 Generation Exhibiting a Positive Antibody Response
 (Titer \geq 20) to Inoculated MCF-247 Virus

<u>Cross</u>	<u>H-2^{b/d} haplotype</u>	<u>H-2^{d/d} haplotype</u>
F ₁	6/6	0/0

Table 7.4

Observed H-2 Haplotypes of the (C57BL/6J X DBA/2J)F₂
 Generation and the (C57BL/6J X DBA/2J) X DBA/2J Backcross
 Generation Exhibiting a Positive Antibody Response
 (Titer \geq 20) to Inoculated MCF-247 Virus

<u>Cross</u>	Number of mice with positive antibody response &		
	<u>H-2^{b/b} Haplotype</u>	<u>H-2^{b/d} Haplotype</u>	<u>H-2^{d/d} Haplotype</u>
F ₂	5/26	4/26	10/26
Backcross to DBA/2J	--	13/44	14/44

Table 7.5

Chi Square Analysis of the Association of the
H-2 Major Histocompatibility Complex with the Humoral
Immune Response to Inoculated MCF-247 Virus in the
(C57BL/6J X DBA/2J)F₂ Generation

Number of Mice with positive antibody
response (Titer \geq 20) and

	H-2b/b Haplotype	H-2b/d Haplotype	H-2d/d Haplotype	Total
Observed number	5	4	10	19
Expected number	6	13	0	19
(Obs. - Exp.)	-1	-9	10	0
(Obs. - Exp.) ²	1	81	100	
(Obs. - Exp.) ² /Exp.	0.1667	6.2308	∞	∞

X² from calculations: ∞

From the Chi Square Table, X²_{0.05, 2} : 5.991

Table 7.6

Chi Square Analysis of the Association of the H-2 Major Histocompatibility Complex with the Humoral Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Generation

Number of Mice with positive anti-body response (Titer \geq 20) and

	H-2b/d Haplotype	H-2d/d Haplotype	Total
Observed number	13	14	27
Expected number	27	0	27
(Obs. - Exp.)	-14	14	0
(Obs. - Exp.) ²	196	196	
(Obs. - Exp.) ² /Exp.	7.2593	∞	∞

χ^2 from calculations: ∞

From the Chi Square Table, $\chi^2_{0.05,1}$: 3.841

Table 8.1

Potential Gametes Generated and Expected Genotypic
and Phenotypic Ratios for Coat Color Resulting
From a (C57BL/6J X DBA/2J) X (C57BL/6J X DBA/2J) Cross

Coat color loci (Both parents): b^+b

Potential Gametes Formed: b^+ , b

Punnett Square for Cross

	b^+	b
b^+	b^+b^+	bb^+
b	bb^+	bb

Expected Phenotypic Ratios:

3 black: 1 brown
(responder):(nonresponder)

Table 8.2

Potential Gametes Generated and Expected Genotypic and Phenotypic Ratios for Coat Color Resulting From a (C57BL/6J X DBA/2J) X DBA/2J Backcross

Coat color loci
(C57BL/6J X DBA/2J) Parent: b^+b

DBA/2J Parent: bb

Potential Gametes Formed
(C57BL/6J X DBA/2J) Parent: b^+, b

DBA/2J Parent: b

Punnett Square for Cross

	b
b^+	b^+b
b	bb

Expected Phenotypic Ratios:
1 black: 1 brown
(responder):(nonresponder)

Table 8.3

Coat Colors of (C57BL/6J X DBA/2J)F₂ Mice and
 (C57BL/6J X DBA/2J) X DBA/2J Backcross Mice Showing a
 Positive Antibody Response (Titer \geq 20) to
 Inoculated MCF-247 Virus

cross	Number of Mice with positive Ab response and		
	black coat color gene(s)	brown coat color genes	Total
F ₂	18/32	6/32	24/32
Backcross to DBA/2J	17/44	10/44	27/44

Table 8.4

Chi Square Analysis of the Association of the Coat Color Gene b with the Humoral Immune Response to Inoculated MCF-247 Virus in the (C57BL/6J X DBA/2J)F₂ Generation

	Number of mice with positive antibody response (titer \geq 20) and		
	black coat color gene(s)	brown coat color genes	Total
Observed number	18	6	24
Expected number	24	0	24
(Obs. - Exp.)	-6	6	0
(Obs. - Exp.) ²	36	36	
(Obs. - Exp.) ² /Exp.	1.3333	∞	∞

X² from calculations: ∞ X²_{0.05,1} : 3.841

Table 8.5

Chi Square Analysis of the Association of the Coat Color Gene b With the Humoral Immune Response to Inoculated MCF -247 Virus in the (C57BL/6J X DBA/2J) X DBA/2J Backcross Generation

	Number of mice with positive antibody response (titer \geq 20) and		
	<u>black coat color gene(s)</u>	<u>brown coat color genes</u>	<u>Total</u>
Observed number	17	10	27
Expected number	27	0	27
(Obs. - Exp.)	-10	10	0
(Obs. - Exp.) ²	100	100	
(Obs. - Exp.) ² /Exp.	3.7037	∞	∞

X² from calculations: ∞

X²_{0.05,1} : 3.841

DISCUSSION

The murine immune response to MCF viruses is of interest because it may serve as a possible way to regulate these viruses in vivo. After our initial strain survey indicated that most strains of mice mount a humoral immune response to inoculated MCF-247 virus, we attempted to determine what controls this response by a genetic study. For the genetic study, the C57BL/6J strain (hereafter referred to as B6) and the DBA/2J strain (hereafter referred to as D2) were chosen as the parental strains. Since the B6 was a very high responder to the inoculated MCF-247 virus while the D2 was almost a complete non-responder, we felt that the genetics of the immune response controls in this strain combination could be easily followed. The fact that these mice differ at many genetic loci which can be followed to map newly discovered genes was an added attraction.

An indirect immunofluorescence assay was used for testing for antibody titers against inoculated MCF-247 virus. The tests revealed that the B6 strain produced a high antibody titer (> 160) in response to the virus while the D2 strain did not respond (titer ≤ 10). The F₁ generation of the B6 X D2 cross was found to uniformly

produce antibody to the virus with a titer > 80 . From this it could be concluded that the immune response is a dominant trait. Studies of the Backcross to D2 and F_2 generations showed that the response segregated into responders and nonresponders. Thus, the response appeared to be genetically controlled.

Data from the assays indicated three phenotypes: nonresponders, low responders, and high responders. Various models were proposed to explain the genetic control of the immune response and Chi Square analysis was used to evaluate each model. The first model proposes that one dominant gene controls the response. For this model, all the responders are placed in one class and all the nonresponders in another. From this model it would be expected that the phenotypic ratio in the backcross mice would be 1:1 and the phenotypic ratio in the F_2 mice would be 3:1. Indeed, it was found that the backcross and F_2 data using a titer ≥ 20 for a positive response fit this one gene model very well.

A two gene model allowing for both high (titer ≥ 80) and low ($20 \leq$ titer < 80 or $40 \leq$ titer < 80) responders was also proposed. From this model it would be expected that the phenotypic ratio for the backcross mice would be 1 high responder:2 low responders:1 non-responder and the phenotypic ratio for the F_2 mice would be 9 high responders:6 low responders:1 nonresponder. Chi Square analysis of the backcross and F_2 data did

not support this model. Another two gene model requiring the presence of two dominant genes for an immune response was proposed. The expected phenotypic ratios for the backcross mice, according to this model, would be 1 responder:3 nonresponders. For the F₂ generation it would be 9 responders:7 nonresponders. Chi Square analysis of the backcross data did not support this model. However, the model was supported by F₂ data using both a titer ≥ 40 and titer ≤ 80 as cutoff points for a positive response.

On the basis of the Chi Square analyses, it appears most likely that one gene is involved in regulating the humoral immune response to MCF-247 virus. However, the possibility of 2 genes being involved cannot be excluded. It would be interesting to obtain more mice and conduct further progeny testing to discriminate further between the two possibilities.

The possible involvement of the H-2 major histocompatibility complex with the humoral immune response was investigated. Studies conducted throughout the 1970's have found that this complex (located on mouse Chromosome #17) has a region within it, called the I (Immune Response) Region, which controls a variety of immune phenomena such as transplantation antigens, differences in level of antibody response to many antigens and differences in susceptibility to tumor viruses (12). Involvement of the H-2 Complex with the humoral immune

response to MCFs would provide new information about the possible function of this complex in relation to infection by recombinant RNA viruses. An understanding of a possible H-2 linked immune response mechanism would also be of concern here in view of its possible role in explaining the association between the human histocompatibility complex and various human leukemias.

Hemagglutination tests were done on erythrocytes from backcross and F₂ mice in order to determine the H-2 haplotypes of the responders and nonresponders. Knowing that the haplotype of the B6 parent is H-2^b and the haplotype of the D2 parent is H-2^d, it was expected that any F₂ or backcross mouse showing a positive antibody response (titer ≥ 20) would have at least one H-2^b allele. Such was not the case in our studies, and Chi Square analysis showed no linkage of the H-2 complex with the immune response. The response to MCF-247 virus appears to be similar to that to type III pneumococcal polysaccharide, in which non-H-2 genes control the immune response (13).

The coat color gene b, responsible for determining whether the coat color will be black or brown, was followed with the hope that it would provide information for the mapping of the immune response gene(s) expressed in response to the presence of MCF-247 virus. The B6 mice have the b⁺b⁺ genotype, and the D2 mice have the bb genotype. Since the F₁ mice uniformly produced a positive

antibody response (titer > 80) to the MCF-247 virus, it was felt that the genes may be linked. However, data for the F₂ and backcross mice strongly disagreed with this hypothesis, since both black and brown mice responded positively. We have concluded that there is no association between the coat color gene and the immune response gene(s).

This study has provided interesting information about the humoral immune response to MCF-247 virus, but much more work is indicated to fully understand this response. The number of genes involved needs to be further elucidated. Mapping of the gene(s) involved remains to be done. In addition, it would be very helpful to determine which class of immunoglobulin is produced in response to the virus. Further research should prove challenging and rewarding and could produce information helpful in understanding the association between the human immune system and various human leukemias.

LITERATURE CITED

1. Tooze, J., ed. 1973. The Molecular Biology of Tumor Viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. McKenzie, K.F.C., T. Potter. 1979. Murine lymphocyte-surface antigens. *Adv. Imm.* 27:273.
3. Lerner, R.A. 1978. Recombinant origins of leukemogenic murine viruses. Parke-Davis Award Lecture. *Am. J. Path.* 93(1):9-20.
4. Chattopadhyay, S.K., M.W. Cloyd, D.L. Linemeyer, M.R. Lander, E. Rands, and D.R. Lowy. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. *Nature (London)* 295(5844):25-31.
5. Elder, J.H., J.W. Gautsch, F.C. Jensen, R.A. Lerner, J.W. Hartley, and W.P. Rowe. 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (env) gene recombinants. *Proc. Nat. Acad. Sci. US* 74(10):4676-4680.
6. Weiss, R.A., N.M. Teich, H.E. Varmus, J.N. Coffin, eds. 1982. *Molecular Biology of Tumor Viruses*, 2nd Edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
7. Cloyd, M.W., J.W. Hartley, and W.P. Rowe. 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. *J. Exp. Med.* 151:542-545.
8. Hartley, J.W., N.K. Wolford, L.J. Old, and W.P. Rowe. 1977. A new class of murine leukemia virus associated with the development of spontaneous lymphomas. *Proc. Nat. Acad. Sci. US* 74(2):789-792.
9. Cloyd, M.W., J.W. Hartley, and W.P. Rowe. 1981. Genetic study of lymphoma induction by AKR mink cell focus-inducing virus in AKR X NFS crosses. *J. Exp. Med.* 149:702-712.

10. Cloyd, M.W., J.W. Hartley, and W.P. Rowe. 1979. Cell-surface antigens associated with recombinant mink cell focus-inducing murine leukemia viruses. *J. Exp. Med.* 149:702-712.
11. Ruscetti, S., J. Feild, L. Davis, and A. Oliff. 1982. Factors determining the susceptibility of NIH Swiss mice to erythroleukemia induced by Friend murine leukemia virus. *Virology* 117:357-365.
12. Schreffler, D.C. and C.S. David. 1975. The H-2 Major Histocompatibility Complex and the I Immune Response Region: genetic variation, function and organization. *Adv. Imm.* 20:126.
13. Amsbaugh, D.F., C.T. Hansen, B. Prescott, P.W. Stashak, D.R. Barthold, and P.J. Baker. 1972. Genetic control of the antibody response to type III pneumococcal polysaccharide in mice. *J. Exp. Med.* 136:931-949.

RECEIVED
JAN 11 1980