A new histocompatible-2 mouse recombinant haplotype and the response of this recombinant to antigen Ea-2.1

by Eugene Alexander Johnson

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University

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Nov 4, 1975
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Abstract

The ability of inbred mouse strains to respond to the histocompatible antigen Ea-2.1 appears to be regulated by the major Histocompatibility-2 gene complex. An H-2 recombinant mouse, using a responder strain B10.RIII as one of the parental types, was developed and tested for Ea-2.1 response. The cellular and humoral response to Ea-2.1 antigen exhibited by the recombinant was different from either parental haplotype. A two-gene Ea-2.1 antigen responder system is proposed as a possible explanation for the observed data.

Introduction

The H-2 histocompatibility system comprises that portion of the chromosome segment between and including H-2K and H-2D genetic subregions. The system was first described by Peter Gorer in 1936 (1937) and placed in linkage group IX (chromosome 17) (Hoecker 1959). The terminology, rules and proposed definitions used in this paper are in accord with Klein et al. (1974a). There are four main regions of the H-2 complex; K, I, S and D marked by the corresponding genes H-2K, Ir-1, SsSlp and H-2D (Figure 1). Another region is proposed by David, Stimpfling and Shreffler (1975c) and Klein (1975). The region is thought to lie between the S and D regions and is defined as H-2G. The H-2G region determines the H-2.7 antigen (Figure 1). Figure 1 also shows the distribution of histocompatibility antigens, according to their responsible regions, of the mice used in this study.

The respective regions have been demonstrated by intra-H-2 recombinations. The I region includes the subregions Ir-1A, Ir-1B and Ir-1C (David and Shreffler 1975a, Benacerraf and Katz 1975, David et al. 1975b). See Figure 1. The K-end is to the left of the Ss region and proximal to the centromere (Pizarro and Dunn 1970). The D-end is right of Ss and left of TL. The variant forms of each subregion are provisionally termed alleles. Combinations of specific

alleles at each of the subregions within the H-2 complex associated with a given chromosome are called haplotypes. Each haplotype is denoted as H-2 with a letter superscript. As an example, strain C57BL/10 (abbreviated B10) has the $H\text{-}2^b$ haplotype. The respective haplotypes are defined by prototype strains. Recombinant haplotypes occurring more than once within given parental haplotypes are noted by a numeral following the superscript letter. As an example, $H\text{-}2^{t2}$ haplotype is the second recombination between haplotypes $H\text{-}2^d$ and $H\text{-}2^s$ (Table 2).

The Ea-2 cellular antigen system. A cellular antigen system reported to play a role in the allograft reactions of mice is determined by the Ea-2 locus. Ea-2, also called R-Z and H-14 is a codominant allelic system first described by Hoecker et al. (1959). The allele $Ea\text{-}2^a$ is responsible for the antigen Ea-2.1, also denoted R, while $Ea\text{-}2^b$ is responsible for antigen Ea-2.2, also called Z. Animals heterozygous at the Ea-2 locus express both antigens. The specificity is found on erythrocytes and other tissue cells (Popp 1967). Snell and co-workers (1967) demonstrated that the responsible genes were allelic and assorted independently of the major histocompatibility system H-2. This work was later supported by a study done by Popp (1969). Hoecker

and Pizarro (1961) presented data suggesting that Ea-2 was linked to H-2. Later work done by Pizarro and Vergara (1973) supported this view. Reasons for the disparity are not clear but may be due to a chromosomal translocation within the strain RIII colony of Hoecker and co-workers or the use of inadequately defined serotyping reagents (Stimpfling 1974).

All mouse strains tested, thus far, carry the Ea-2.2 (Z) antigen associated with the $Ea\text{-}2^b$ allele except those that possess the Ea-2.1 (R) antigen associated with the $Ea\text{-}2^a$ allele. To date, no inbred strain is known to lack or possess both antigens. The antigens are easily detected via alloantisera in a variety of tests such as hemagglutination (HA), trypan blue exclusion cytotoxic (CT) and leucoagglutination.

The strains expressing the Ea-2.1 antigen are RIII/Wy, F/St, RF/J and RFM/Um. In addition there are seven congenic lines in which the $Ea\text{-}2^a$ haplotype was transferred to C57BL/6, C57BL/10 or C57BL/Ha genetic backgrounds from either RIII/Wy or F/St strains (Stimpfling 1974, Strong 1942, Furth et al. 1933, Popp and Amos 1965, Popp 1967).

The following strains of mice were used in the experiments reported in this paper; B10.RIII, B10.RIII(R), B10.RIII(Rr), B10.S(7R) and C57BL/10 (B10) (Table 1 and

Figure 2). Strains B10.RIII and B10.RIII(R) were derived from an outcross of strain RIII/Wy to C57BL/10. The hybrids and selected backcross hybrids were backcrossed repeatedly to a B10 parent with selection for the $\underline{H\text{-}2^r}$ and $\underline{Ea\text{-}2^a}$ haplotypes of the RIII parent. After nine generations of backcrossing to the recurrent B10 parent, the $\underline{H\text{-}2^r}$ and $\underline{Ea\text{-}2^a}$ haplotypes were separated into two sublines. Strain B10.RIII is congenic with B10 except for an $\underline{H\text{-}2}$ difference, while B10.RIII(R) is congenic with B10 except for an $\underline{Ea\text{-}2}$ difference. Simular lines were produced by G. D. Snell at the Jackson Laboratory and designated B10,RIII(71NS) and B10.RIII(72NS) corresponding to strains B10.RIII and B10.RIII(R), respectively (Stimpfling 1974).

Strain B10.RIII(Rr) was derived by mating B10.RIII to B10.RIII(R) mice. The F_1 hybrids were mated to yield offspring homozygous for both $\underline{H\text{-}2^r}$ and $\underline{Ea\text{-}2^a}$ as well as the heterozygous combinations. Those animals that were homozygous for both genes were interbred to establish a line congenic with B10 except for differences at the $\underline{H\text{-}2}$ and $\underline{Ea\text{-}2}$ loci.

Strain B10.S(7R) is a recombinant line congenic with B10 except for the substitution of $\underline{H\text{-}2^{t2}}$ for the $\underline{H\text{-}2^b}$ haplotype of B10. $\underline{H\text{-}2^{t2}}$ is derived from a crossover within the

H-2^a and H-2^s haplotypes and possesses the H-2D region of H-2^a and the H-2K region of H-2^s (Table 2).

Response to the Ea-2 gene products. Whether or not the Ea-2 system elicited an antibody response or an allograft reaction depends on the mouse strains or hybrids being challenged. Reciprocal immunizations of B10 and B10.RIII(R) mice do not produce either an anti Ea-2.1 or Ea-2.2 antibody response. Skin grafts are reciprocally accepted. However, (LP X B10.RIII)$_{F_1}$ and (B10 X B10.RIII)$_{F_1}$ hybrids respond vigorously to B10.RIII(R) tissue. A high titer of HA antibody against Ea-2.1 is produced and skin grafts from B10.RIII(R) mice to (LP X B10.RIII)$_{F_1}$ hybrids are rapidly rejected (Stimpfling 1972, unpublished data) (Table 5). Strain LP shares the H-2^b haplotype with strain B10 but differs at other histocompatibility loci. B10.RIII and B10.RIII(R) are presumably congenic except at the H-2 and Ea-2 loci. B10.RIII is H-2^r; Ea-2^b and B10.RIII(R) is H-2^b and Ea-2^a. Consequently the (LP X B10.RIII)$_{F_1}$ hybrid is histocompatible with B10.RIII(R) skin grafts except for the Ea-2 difference. (LP X B10)$_{F_1}$ hybrids do not produce anti-Ea-2.1 antibodies or reject B10.RIII(R) skin grafts in response to the Ea-2 difference. From these data it was evident that the H-2^r haplotype of B10.RIII regulates the

immune response to the Ea-2.1 antigen.

Mapping of the immunoregulator gene that controls the Ea-2.1 response. The ability of the $H\text{-}2^r$ haplotype to regulate the Ea-2.1 response was further investigated. Three recombinants involving the $H\text{-}2^r$ haplotype have been identified but two of them were not preserved (Stimpfling 1974). Since the H-2 complex can be divided into several genetically distinct regions (Figure 1) recombination analysis was undertaken to map the region or regions controlling the response to the Ea-2.1 antigen.

Materials and Methods

Care of mice. All mice were fed and watered ad libitum using Purina Mouse Chow and tap water. The mice were kept in plastic cages with wood shavings as bedding. The cages were changed weekly. All mice were pedigreed and ear marked at weaning. Records were kept of each mating. All mice were reared in the animal colony of the McLaughlin Research Institute in Great Falls unless otherwise noted. All strain designations prefaced with B10 denote a C57BL/10 genetic background.

Selection of mouse strains for recombination analysis. Strain B10.S(7R) was selected to cross with strain B10.RIII. B10.S(7R) is a strain developed by Dr. Stimpfling and carries a recombinant chromosome designated H-2^{t2} (Figure 2 and Table 2, Stimpfling and Reichert 1970). B10.S(7R) has easily detected private antigens determined by both the H-2D and the H-2K regions of the H-2 gene complex. Private antigens are usually restricted to a single H-2D or H-2K allele while public antigens are shared with two or more alleles. Two of the private antigens, H-2.4 and H-2.19, possessed by B10.S(7R) are not shared by either the H-2^{r} haplotype of B10.RIII or the H-2^{b} haplotype of strain B10. [B10.S(7R) X B10.RIII]$_{F_1}$ hybrids were testcrossed to B10 mice and the testcross offspring were serotyped at about seven weeks of

age. Typing was accomplished using alloantisera specific for H-2 antigens H-2.4, H-2.19 and H-2.2 (Tables 3 and 4). Figure 3 illustrates the abbreviated H-2 systems of the experimental animals. Mice were typed by the PVP HA method of Stimpfling (1961) using thrice washed erythrocytes obtained from tail bleedings of the testcross progeny.

Preparing the alloantisera. Alloantisera were prepared according to Stimpfling et al. (1964) in appropriate donor recipient combinations (Tables 3 and 4). Hybrids were often used to produce reagents having the desired specificity. The donor and recipient strains were selected on the basis of minimal alloantigen differences to produce a monospecific antiserum, if possible. Cell suspensions were prepared from aseptically removed spleen, thymus and the inguinal and axial lymph nodes by passing the tissues through a cytosieve (Snell 1953). The cells were suspended in Hanks' Basic Salt Solution. A ratio of one donor animal per 15 recipients was used. Three immunizations were administered IP at weekly intervals. During the fourth week the hyperimmunized animals were bled from the tail and two days later bled again. Seven days later the recipients were again immunized and the bleedings repeated as before. This regimen was repeated until the animals were bled 10 to 12 times. The final

bleeding was done retro-orbitally and the animals were exsanguinated. Each antiserum pool was tested against a standard panel of erythrocytes consisting of the animals shown in Table 4. The resulting titers of HA activity were made a part of the record for that serum. The sera used to type the testcross offspring are listed in Tables 3 and 4.

Typing the testcross progeny for recombination. Using the antisera listed in Table 3 the testcross offspring were tested for antigens H-2.4 and H-2.19. Progeny that were heterozygous $\underline{H\text{-}2^{b}}/\underline{H\text{-}2^{t2}}$ were positive for H-2.4 and H-2.19 derived from the B10.S(7R) parent. The progeny that were heterozygous $\underline{H\text{-}2^{b}}/\underline{H\text{-}2^{r}}$ were expected to be negative for both of the antigens. Both heterozygous types were positive for antigen H-2.2, the private antigen associated with $\underline{H\text{-}2^{b}}$ from the B10 parent. Using these antigens as markers, testcross progeny were serotyped to find an animal positive for only one of the $\underline{H\text{-}2^{t2}}$ derived antigens H-2.4 or H-2.19. The B10 derived marker, H-2.2, was used later to type for homozygosity of the recombinant haplotype. All testcross progeny were positive for H-2.2 including a possible recombinant. The recombinant, when it appeared, would be positive for H-2.4 and negative for H-2.19 or vice versa, indicating a probable crossover event between H-2D and H-2K ends of the

two parental haplotypes. The map distance between the H-2K and H-2D regions is estimated to be 0.5 map units (Figure 1) (Stimpfling 1971, Shreffler 1970). A crossover event could be expected in the ratio of 1/200 testcross progeny. Since the frequency of crossing over is higher in oogenesis, female [B10.RIII X B10.S(7R)]$_{F_1}$ hybrids were preferred for the testcross mating (Ibid, Ibid). About twenty breeding pairs were maintained throughout the experiment.

Establishing a new recombinant line. A total of 402 testcross progeny were serotyped. A male, number 58559, was found to be H-2.4 negative and H-2.19 positive. He was mated with two females of strain B10 in a backcross progeny test. These matings yielded 42 backcross progeny. Twenty-one had a serotype corresponding to that of the variant male while twenty-one were homozygous for the H-2^b haplotype. The data confirmed the genetic nature of the variant. The new haplotype was given the designation B10.RIII(20R). Sib-sib matings between backcross hybrids heterozygous for the 20R and H-2^b haplotypes produced 74 offspring. Serotyping these animals revealed 25 heterozygotes H-2^b/20R, 25 homozygotes 20R/20R and 24 homozygotes H-2^b/H-2^b. The data deviates significantly from the expected 1:2:1 ratio ($P > 0.1$).

The 20R homozygotes were then tested for other H-2 specificities. Testing was done by hemagglutination (HA) screening, as previously described, (Tables 3 and 4) and by cytotoxic tests (CT) (Figures 4, 5 and 6). CT tests were done as described by Boyle (1968) with some modifications (Johnson 1973). Animals to be typed were bled from the tail into heparin coated tubes. The blood was diluted with phosphate buffered saline (PBS) and the leucocytes separated on a Hypaque-Ficoll gradient. The washed leucocytes were treated with an ammonium chloride solution to clear away transient erythrocytes and again washed. The leucocytes were incubated with antiserum and then rabbit complement was added in a two step procedure. Rabbit complement was obtained from rabbits kept at the Institute. Rabbit donors were tested for a low level of natural anti-mouse cytotoxic activity and a high level of complement activity routinely. A natural anti-mouse CT activity of less than 10% at a dilution of 1:10 in PBS gave an acceptable background in the cytotoxic tests. Complement activity was determined by using serial dilutions of the rabbit sera in a CT test with an alloantiserum of known high titer. A complement mediated cell kill of greater than 50% at a dilution 1:20 in PBS was indicative of a good positive control. Complement was

stored at minus 20°C and the stock was discarded when six weeks old. Each CT test was done with both a positive control (cells positive for the antiserum) to insure that the complement was active and a negative control (cells without alloantisera) to insure that the complement did not have non-specific anti-mouse activity. Three of 11 rabbits tested in the colony could supply complement to meet these standards. Assay of CT activity was by trypan blue dye exclusion.

The alloantisera were prepared in various hosts using tissue as the immunogen (Table 3) by the method previously described. All antisera were stored at minus 20°C.

In vivo adsorption. In vivo adsorption was accomplished by injecting animals intraperitoneally with 0.2 ml of the antisera and bleeding three hours later. A second bleeding was done 24 hours later; however, this second pool was consistently low in CT antibody titer and the second bleeding was discontinued.

Skin grafting. Skin grafts were done according to the procedure of Silmser et al. (1955). Animals to be grafted are anesthesized with 0.01 ml of chloral hydrate per gram of body weight. This is administered IP simultaneously with a prophylactic injection of penicillin. A graft bed is

prepared on the dorsal surface of the thorax by shaving and then excising a portion of skin. The donor skin tissue is taken fresh from the tail of the donor animal. The graft is covered by a shield made from a heat molded cellulose acetate cover slip and held in place with cellulose tape. The graftees are monitored for 10 to 11 days and bandages that need tending are repaired or replaced. On the 11th day, post operative, the bandages are removed and the dead epidermis carefully soaked with PBS and peeled off. Removal of the ghost skin exposes the viable tail skin which can be evaluated for signs of rejection or acceptance. Grafts were monitored for as long as 200 days or until rejected.

Results

Ss and Ia typing. B10.RIII(20R) mice were sent to Dr. Chella David, of the University of Michigan, for determination of I and S region specificities. The variant has both the I and S regions of $H\text{-}2^{t2}$, thus establishing the probable occurrence of a crossover between the Ss and H-2D regions of the H-2 complex.

Serological data. The results of hemagglutination tests (Table 4) clearly indicate that the haplotype of the variant differs from both $H\text{-}2^{t2}$ and $H\text{-}2^{r}$. The haplotype of the variant, B10.RIII(20R), has lost antigens H-2.4 and H-2.13 associated with the H-2D region of $H\text{-}2^{t2}$ but retained H-2.19 and H-2.7. H-2.19 and H-2.7 are associated with the H-2K and H-2G subregions of $H\text{-}2^{t2}$, respectively. The data are in accord with the findings of David and co-workers (1975c) and Klein (1975) with regard to the H-2G subregion and further established the point of probable crossing over to be between H-2D and H-2G (Figure 3).

Cytotoxic tests. The results of the cytotoxic tests, likewise, indicate that B10.RIII(20R) is unlike $H\text{-}2^{r}$ with respect to lymphocyte antigens. SAS-346 (Figure 6), an alloantiserum made in $[\text{RIII X B10}]_{F_1}$ hybrids using B10.RIII(20R) as the immunogen, produced a high titer of antibody against $H\text{-}2^{s}$, $H\text{-}2^{t2}$ and the donor lymphocytes. The complexity of the lymphocyte antigens associated with the I subregions

was recently described by David and Shreffler (1975a). One or more of the antigens detected by SAS-346 may correspond to antigens of the I complex. SAS-346 also had a high titer of HA antibody directed against H-2^s, H-2^{t2} and the recombinant. The positive reactions with H-2^f and H-2^{t4} offer strong evidence that both anti H-2.19 and H-2.7 are present. H-2^f lacks H-2.19 as does H-2^{t4}. Failure to agglutinate either H-2^a or H-2^d cells is consistent with a lack of anti H-2.4 activity. The lack of response against H-2^b is significant in that the recombinant is clear of detectable HA histocompatibility antigens associated with H-2^b. The serum was not active against H-2^r as expected.

Alloantiserum was produced in B10.S(7R) animals using B10.RIII(20R) tissue (SAS-293: Figure 5). In this combination the donor and recipient differ at only the H-2D subregion. The antiserum has no detectable CT antibody directed against either H-2^r or against the variant lymphocytes. Alloantiserum produced in H-2^{t2} recipients against H-2^r showed no cross reactivity against H-2^s, H-2^{t2} or the variant (Figure 4). HA testing of SAS-293 revealed a very low transient titer of hemagglutinating antibody against B10.RIII(20R) and H-2^b but it was negative for H-2^r. The failure to demonstrate an erythrocyte antigen associated

with the H-2D end of $H\text{-}2^r$ is consistent with other findings. Earlier efforts to demonstrate a private erythrocyte antigen associated with the $H\text{-}2^r$ haplotype were not successful. In only four of ten different antisera produced against $H\text{-}2^r$ was there any HA activity; and in these four antisera there was a broad range of cross reactivity with other haplotypes corresponding to the private H-2K subregion antigens H-2.11 and H-2.25. $H\text{-}2^r$ possesses the private antigen H-2.18 tentatively placed in the H-2D subregion (Klein 1975a); however, the failure to demonstrate any strong CT or HA activity against $H\text{-}2^r$, when the variant was used as the tissue donor in a serum produced in the other parental haplotype, $H\text{-}2^{t2}$, suggests that the determinant of H-2.18 may be situated to the left of H-2D.

Alloantisera produced in $[DBA/2 \times B10.F]_{F_1}$ hybrids against B10.RIII tissue (SAS 282; Figure 7) has a high titer of activity against both parental haplotypes, $H\text{-}2^r$ and $H\text{-}2^{t2}$, the variant and $H\text{-}2^s$ lymphocytes. No activity was detected against $H\text{-}2^b$ haplotype as the results with strain B10 indicate. This serum was adsorbed *in vivo* in strain B10.S(7R) animals and tested against both parental haplotypes $H\text{-}2^r$ and $H\text{-}2^{t2}$, the variant and $H\text{-}2^s$. The results of this test offered positive evidence that the variant shares an antigen

with the H-2^r haplotype since adsorption in H-2^{t2} cleared for H-2^{t2} and H-2^s but not for H-2^r and the variant (Figure 8). Hemagglutination, Ia and Ss typing established that the variant was identical to the H-2^{t2} haplotype in the H-2G, Ss, Ia and H-2K regions. Therefore; this residual activity, noted in the SAS 282 adsorption test, which the variant shares with H-2^r must be associated with the chromosome to the right of H-2G. On the basis of these data the variant is postulated to be a recombinant involving the parental haplotype H-2^{t2} to the left of H-2D and the parental haplotype H-2^r to the right of H-2G.

Grafting data. All grafts were done as previously described; with care taken to preclude the placing of male tail skin on female recipients. Females were often used as donors for male recipients; however, there is no evidence that intrastrain female to male skin grafts are ever rejected.

If B10.RIII(20R) is indeed a recombinant, neither of the parental types should accept tissue grafts from the recombinant or vice versa. Table 5 shows the total rejection of all reciprocal skin grafts between B10.RIII and the recombinant and the same for B10.S(7R) and the recombinant. These data precluded the occurrence of a deletion or loss mutation in the H-2D region of a presumed recombinant.

[B10.S(7R) X B10.RIII]$_{F_1}$ hybrids also received a recombinant strain skin graft. Five of seven of these F_1 hybrids rejected the graft chronically. These results indicate; i. the variant could be a loss - gain mutant, ii. a gain mutation occurred after the recombinant line was established, iii. one of the parental type strains has mutated. The exact reason for this behavior is not clear and may be due to technical reasons not related to histoincompatibility and will be resolved in further study.

Preparation of a B10.RIII(20R) congenic strain. The progeny of sib-sib matings were tested by HA serotyping to insure that segregation was not occurring at the H-2 locus. Seventeen progeny of the first filial generation were tested. Two were found positive for H-2.2 and were discarded. Eighteen second filial generation progeny were tested and all were found to be positive for H-2.19 and negative for H-2.2 and H-2.4. Twenty-seven fourth filial generation animals were tested and all were positive for H-2.19 and negative for H-2.2 and H-2.4. From these animals the recombinant line was established.

One recombinant was found among 402 test cross progeny. The recombination rate for this experiment was about 0.25%. This is slightly lower than expected since the crossover

frequency generally observed is between 0.33% and 0.5%.

Testing the response of the recombinant to antigen Ea-2.1. The recombinant strain, B10.RIII(20R), was hybridized with strain LP/J mice obtained from the Jackson Laboratory at Bar Harbor. The F_1 hybrids were then grafted with tail skin from strain B10.RIII(R) mice. A total of 46 animals were grafted and 14 of the 46 recipients rejected their grafts. The results differed from previous data involving [B10.RIII X LP]$_{F_1}$ hybrids as tissue recipients (Table 5). In the latter case, 42 of 46 animals displayed acute graft rejections. The four animals (10%) accepting the B10.RIII(R) skin grafts were males. All of the females rejected their allografts.

Of the [B10.RIII(20R) X LP]$_{F_1}$ hybrids that received B10.RIII(R) skin grafts, 24 of the males accepted (88%) and three rejected. Of the females, eight of 19 accepted for a 42% acceptance rate. All rejections were acute in both sexes.

The humoral response of [B10.RIII(20R) X LP]$_{F_1}$ hybrids was different from that observed when [B10.RIII X LP]$_{F_1}$ hybrids were immunized with B10.RIII(R) tissue. Alloantiserum produced in [B10.RIII X LP]$_{F_1}$ hybrids exhibit a high titer of HA antibodies specific for the donor Ea-2.1 antigen (Table 4). [B10.RIII X B10]$_{F_1}$ hybrids immunized against

B10.RIII(R) tissue also produce a high titer of anti-Ea-2.1 hemagglutinins (SAS 287; Table 4); however, the hybrid does not reject B10.RIII(R) skin grafts. The B10.RIII(20R) recombinant when hybridized with either strain B10 or LP mice and the F_1 hybrids immunized with B10.RIII(R) tissues produces no detectable humoral response by either hemagglutination or cytotoxic tests.

Discussion

In an effort to map the location of an H-2 linked immune responder gene, a recombinational analysis of the $H\text{-}2^r$ haplotype was undertaken. Ea-2.1, an antigen determined by the $Ea\text{-}2^a$ gene, is immunogenic in only certain inbred strains and hybrid combinations. Strain B10.RIII bearing the $H\text{-}2^r$ haplotype is a responder. A variant derived from a hybrid $H\text{-}2^r/H\text{-}2^{t2}$ heterozygote was identified and was shown to have the H-2K, I, S and H-2G regions of $H\text{-}2^{t2}$, a non-responder haplotype, the H-2D region lacks any detectable antigens. The recombinant was tested for its cellular and humoral response to Ea-2.1 antigen.

When the recombinant, B10.RIII(20R), was crossed with strain LP, the hybrids exhibited a cellular response to B10.RIII(R) skin grafts, a strain positive for antigen Ea-2.1. Thirty-two per cent of the graft recipients acutely rejected the tissue. These data show that mice bearing the recombinant haplotype responded to the skin grafts possessing the Ea-2.1 antigen.

Since only 42% of the $[B10.RIII(20R) \times LP]_{F_1}$ hybrid females accepted the B10.RIII(R) skin grafts versus a 95% acceptance rate displayed by the males, these results may indicate a sex associated phenomenon. That female mice respond to the antigen Ea-2.1 much better than do the males is

not without precedent. Snell (1953) reported a sex associated phenomenon in his review of transplantable tumors. The ability of a tumor to survive was greater in the male host. Wheater and Hurst (1961) noted a similar sex associated response to bacterial infections. Again it was the male mouse that exhibited a higher susceptibility to bacterial pathogens. Snell (1958), Batchelor and Chapman (1965) and Graff and co-workers (1966) noted this type of a response with respect to minor histocompatibility differences. Castro and Hamilton (1972) demonstrated that this phenomenon is related to male hormone. Orchiectomized mice exhibited a response to grafts similar to normal females. Stimpfling and Reichert (1971b) noted a sex associated response related to H-2 haplotypes. Since their study included several H-2 recombinant haplotypes, they were able to propose that the subregion Ir-1 might control the response to Y-linked antigens. It is not uncommon to observe a sexual disparity in response to many different antigens. The data involving rejection of B10.RIII(R) tissue by a larger proportion of the [B10.RIII(20R) X LP]$_{F_1}$ hybrid females than males may exemplify the sex related difference in intensity of immune responses.

In contrast to B10.RIII, the derived recombinant,

B10.RIII(20R), failed to exhibit a humoral response following immunization against Ea-2.1 antigen. Both [B10.RIII(20R) X LP]$_{F_1}$ and [B10.RIII(20R) X B10]$_{F_1}$ hybrids failed to demonstrate a humoral response detectable by either hemagglutination or cytotoxic tests. Humoral response data indicate that strain B10.RIII(20R) is unable to produce anti-Ea-2.1 antibodies, even after hyperimmunization. The inability to respond to Ea-2.1 is shared by B10.S(7R) which possesses the parental haplotype H-2^{t2}. In contrast, B10.RIII has the H-2^{r} haplotype and is able to produce anti-Ea-2.1 antibodies and reject skin grafts possessing Ea-2.1. Since the B10.RIII(20R) recombinant has the H-2D region of the H-2^{r} haplotype, the supposition is that an immune responder gene controlling cellular immunity against antigen Ea-2.1 is to the right of the H-2G subregion in the H-2^{r} haplotype. The recombinant has the H-2^{t2} chromosome to the left of the H-2D region and because H-2^{t2} is a non-humoral responder to antigen Ea-2.1 it appears that a humoral responder gene for Ea-2.1 may lie to the left of the H-2D region of the H-2^{r} haplotype.

Based on these considerations, an Ea-2.1 immune responder is proposed that is comprised of at least two genes. The genes may be closely linked to the H-2 complex as in

the 2-gene immune responder system for the synthetic terpolymer GL-Φ described by Dorf and co-workers (1975) and the 2-gene system reported by Taussig and Munro (1975). Or they may be loosely linked as in the Thy-1.1 2-gene responder system reported by Zaleski and Klein (1974) in which one gene mapped within the H-2 complex and the second was 17.1 map units to the right of the H-2 complex. A more extensive linkage study would be required to establish the precise positions of the proposed Ea-2.1 immune responder genes in relation to the H-2 complex.

The humoral Ea-2.1 antigen responder gene is proposed to lie left of the H-2G subregion. The putative locus includes two alleles and consists of a dominant responder allele, provisionally called Ir-HR, and a recessive non-responder allele provisionally called Ir-Hr. The proposed cellular Ea-2.1 immune responder gene locus lies to the right of the H-2G subregion and includes a pair of alleles. This locus can contain either the dominant non-responder allele provisionally called Ir-CN or the recessive Ea-2.1 responder allele provisionally called Ir-Cn. The regulatory function of these two genes can be independent as shown by the $[B10.RIII(20R) \times LP]_{F_1}$ hybrids which can exhibit only a cellular response to Ea-2.1; and the $[B10.RIII \times B10]_{F_1}$

hybrids which can exhibit only a humoral response to the antigen. In the case of the $[B10.RIII \times LP]_{F_1}$ hybrids both humoral and cellular responses are observed. Conversely, the $[B10 \times LP]_{F_1}$ and $[B10.S(7R) \times LP]_{F_1}$ hybrids produce neither response.

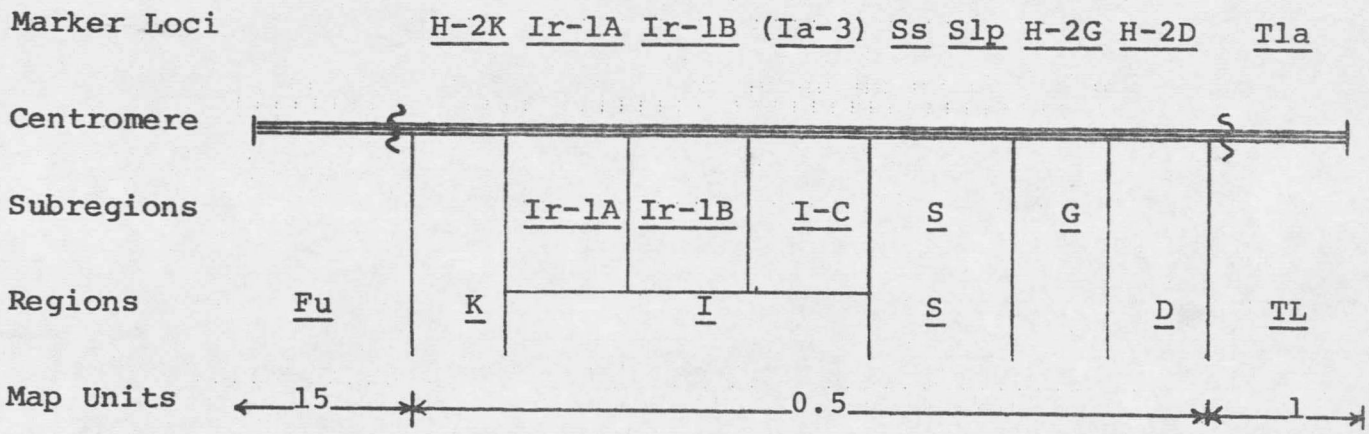
Assigning the presumed alleles Ir-HR and Ir-Cn to the $H\text{-}2^r$ (B10.RIII) haplotype and Ir-Hr and Ir-Cn to the $H\text{-}2^{bc}$ (LP) haplotype a hybrid of these two haplotypes would be heterozygous dominant for a Ea-2.1 humoral response gene and homozygous recessive for a Ea-2.1 cellular response gene (Figure 7). Presumably the $H\text{-}2^b$ (B10) haplotype carries the Ir-Hr and Ir-CN alleles. This haplotype when hybridized with B10.RIII ($H\text{-}2^r$) would result in a hybrid that is heterozygous dominant for a Ea-2.1 humoral response gene and heterozygous dominant for a Ea-2.1 non-cellular response gene. Support for this configuration is observed when $[B10.RIII \times LP]_{F_1}$ hybrids are test crossed to B10. None of these test cross progeny make a cellular response to Ea-2.1 but 50% of them do make a humoral response to the antigen. When $[B10 \times LP]_{F_1}$ hybrids are challenged with Ea-2.1, neither a humoral nor a cellular response is elicited since this hybrid would be homozygous recessive for the Ir-Hr allele and heterozygous dominant for the IrCN/Ir-Cn alleles.

The recombinant, B10.RIII(20R), carries the provisional Ir-Hr and Ir-Cn alleles derived from the $\underline{H\text{-}2^{t2}}$ and $\underline{H\text{-}2^{r}}$ haplotypes respectively. When hybridized with $\underline{H\text{-}2^{bc}}$ the F_1 progeny would be homozygous recessive for the Ir-Hr allele, therefore a non-humoral responder to Ea-2.1, and homozygous recessive for Ir-Cn, the recessive Ea-2.1 cellular responder allele. This proposed 2-gene system for the immune response to antigen Ea-2.1 is consistent with the observed results.

Conclusion

The establishment of a recombinant line of mice derived from the parental haplotypes $\underline{H\text{-}2^r}$ and $\underline{H\text{-}2^{t2}}$ was accomplished in accord with established procedures. The recombinant was then used to ascertain whether or not the I region of $\underline{H\text{-}2^r}$ played a role in the response to the antigen Ea-2.1. The results of this study indicate that the response to Ea-2.1 may be partially due to one or more immuno responder genes located left of the H-2D region in the $\underline{H\text{-}2^r}$ haplotype; and a second immuno responder gene or genes to the right of the H-2G subregion in the $\underline{H\text{-}2^r}$ haplotype. A proposed 2-gene system is offered as one possible explanation for the separation of the humoral and cellular responses observed in selected F_1 hybrids. One immune responder system for humoral response to antigen Ea-2.1 lies left of the H-2D region in the $\underline{H\text{-}2^r}$ haplotype and the second system, for cellular response to the antigen Ea-2.1, lies to the right of the H-2G subregion of the $\underline{H\text{-}2^r}$ haplotype. These responder genes are capable of separate and independent response to Ea-2.1 antigen. They are both alleles and there is at least one dominant and one recessive allele for each of the two loci.

Figure 1. The H-2-TL System on The 17th Chromosome.



From David and Shreffler (1975a) with some modification.