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Regulation of the Production of Murine IgG_{2a} by an *H-2*-Linked Gene and Other Unlinked Genes

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Abstract. Alleles of at least two loci (*rig-1* and *Rig-2*) regulate the levels of serum immunoglobulin of the Igh-1b class and allotype in BALB/c Ig^b (BAB/14) and (BALB/c × BAB/14)F_1 mice. The combined effect of the BALB/c alleles at these two loci is to lower Igh-1b levels significantly below those observed in other strains and below their own levels of Igh-1a in allotype heterozygous mice. The *rig-1* locus is closely linked to or within the *H-2* complex. Two alleles have been defined: *rig-1*^{d} and *rig-1*^{b} in *H-2*^{d} and *H-2*^{b} haplotypes, respectively. Homozygous *rig-1*^{d} animals heterozygous for the BALB/c *Rig-2* allele(s) have very low levels of Igh-1b. The designation of *Rig-2* is provisional since it has not been mapped or defined as a single locus.

Introduction

Studies on the regulation of immunoglobulin levels in mice have largely been restricted to the analysis of perturbations induced by experimental manipulations of otherwise normal animals. Some mouse strains, notably SJL and (BALB/c × SJL) hybrids respond to perinatal exposure to certain anti-immunoglobulin sera by developing a population of suppressor T lymphocytes that suppress the production of certain immunoglobulins. This phenomenon, referred to as "chronic allotype suppression", results in dramatic decreases in the levels of the particular immunoglobulins affected (Jacobson and Herzenberg 1972a, b, Herzenberg et al. 1973, 1975).

Our studies follow the critical observation (Bosma and Bosma 1975, Bosma and Owen 1977) that C.B mice (a BALB/c congenic line carrying the "b" allotype, immunoglobulin heavy-chain genes from a C57BL line) have aberrantly low serum

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levels of Igh-1b. Further experiments by Bosma and co-workers (1977) showed that spleen or myeloma cells from C.B mice producing "b" allotype immunoglobulin do not produce significant amounts of Igh-1b after injection into BALB/c hosts. This suggests that the BALB/c genetic background presents some barrier to the production of Igh-1b.

We found that BAB/14 mice (a sister line to C.B described in the *Materials and Methods* section of this paper) also possess aberrantly low levels of Igh-1b (Dowsett et al. 1977).

Evidence presented here shows that these low levels are caused by regulatory genes at at least two independently segregating loci (or groups of loci). One of these loci (*rig-1*) is closely linked to or within the major histocompatibility complex (*H-2*) in mice.

Materials and Methods

Immunoglobulin terminology. The *IgH* chromosome region codes for the heavy-chain genes of mouse immunoglobulins. Allelic variants (allotypes) within this region are designated Igh^a, Igh^b, Igh^c, etc. The various heavy-chain loci are designated by numbers. For the purposes of this study one should remember that the constant region of γG_{2a} heavy chain is coded by *Igh-1* and the γG_1 heavy chain constant region is coded by *Igh-4*. The designations for the genes and their products have traditionally been used interchangeably, so that, for instance, γG_{2a} of the "b" allotype is referred to as Igh-1b or simply 1b. For this study one should remember that BALB/c mice are "a" allotype for all heavy-chain classes, while C57BL/10, BAB/14, and SJL mice are "b" allotype for all classes.

BAB/14 mice. BAB/14 mice are congenic with BALB/cN but possess the Ig^b allotype, heavy-chain immunoglobulin genes of C57BL mice. The BAB line was donated to the Herzenberg laboratory by Dr. Michael Potter (National Cancer Institute, Bethesda, Maryland, as herterozygous Ig^a/Ig^b BAB/13 mice. One further backcross was made in the Herzenberg laboratory followed by inbreeding and selection of Ig^b homozygous progeny. Hence BAB/14 mice are the product of 14 backcrosses.

Further backcrosses of other mice from the 13th backcross were made by Dr. Potter and others, resulting in the C.B lines of mice. The only known difference between BAB and C.B mice is the result of a genetic crossover which occurred in the BAB line but not the C.B line. This crossover occurred among the structural genes for the V_H region of the immunoglobulin molecule. It results in the fact that BAB/14 mice possess most of the idiotypes of the BALB/cN line and some of the C57BL line, while C.B mice possess primarily C57BL idiotypes (Riblet et al. 1975). This difference has no known effect on allotype suppression as described below.

Quantitative measurements of immunoglobulin levels. For screening large numbers of mice for levels of Igh-1a, Igh-1b, Igh-4a, and Igh-4b, previously described techniques for quantitating immunoglobulin levels were too slow or too cumbersome. For this reason, we have developed a plate-binding assay for quantitating immunoglobulin levels that is both accurate and fast for large numbers of samples (Dowsett 1978).

Briefly, the strategy in this assay is to coat the wells in a 96-well, plastic microtiter plate (Cooke Laboratory Products, Alexandria, Virginia) with a saturating dose of immunoglobulin of a given class and/or allotype (e. g., an Igh-1 a myeloma protein). After incubation to allow binding of the myeloma to the plate and washing to remove free Igh-1a, the wells receive either known quantities of a standard serum or Igh-1a myeloma protein to act as a competitor with the Igh-1a bound to the plate. Each well then receives ^{125}I-labeled, purified anti-Igh-1a in a fixed, limiting concentration. Wells with no Igh-1a added as competitor would bind the maximum bindable fraction of the counts added. Those wells containing competitor Igh-1a would bind fewer counts since the free Igh-1a would bind some of the added counts. The greater the amount of competitor added, the fewer counts would bind to the plate. This, of course, works with all of the other classes and allotypes tested, as well as Igh-1a.

When the logarithm of the amount of myeloma or normal serum standard is plotted against the number of counts bound, a straight line is obtained over a range of 10- to 100-fold, depending on the labeled reagent used. Using this curve, it is possible by interpolation to determine the quantity of the tested class and allotype in the test sera in terms of mg/ml or in percent of normal serum standard.

H-2 typing. A two-stage dye exclusion microcytoxicity test (Amos et al. 1969), as modified (Murphy and Schreffler 1975), was used to establish the *H-2* type of animals. Peripheral blood lymphocytes were used as target cells. They were obtained by bleeding individual mice into 6 × 50 mm tubes containing 0.1 ml sodium heparin (1000 units/ml). The lymphocytes were separated in tubes of the same size on a Ficoll-isopaque gradient. The nucleated cells from this gradient were washed in buffer and spun through an underlayer of FCS at 290 g for 3 min. The pellet so obtained was largely free of platelets. The cells were washed again and adjusted to a concentration of 5×10^5/ml. The tests were performed in microtest tissue culture plates (Falcon Plastics, Oxnard, California).

Statistical method. The Kolmogorov-Smirnov test (Sokal and Rohlf 1969, Miller and Freund 1965) was used to measure the statistical significance of differences between distributions. Since this test is a non-parametric test it does not require that the distributions involved be normal ones. In simple terms, this test measures the difference between the integrals of any two distributions. For comparison of the two distributions where $n_1 \neq n_2$, the two distributions must be normalized to the same area. A shorthand technique for simultaneously normalizing, integrating, and measuring the Kolmogorov-Smirnov statistic can be found in *Selected Tables in Mathematical Statistics* (Harter and Owen 1973). Tables for statistical significance of the statistic are also included for all n's where $10 \leq n_1, n_2 \leq 100$.

Results

Serum Igh-1b levels in BAB/14 mice are controlled by a recessive allele at a locus (rig-1) within or closely linked to H-2. BAB/14 mice have significantly lower levels of serum Igh-1b (1b) than C57BL/10 mice housed with them (see Fig. 1). Since these two lines of mice possess the same immunoglobulin heavy-chain constant-region genes and differ in their genetic background, it is clear that the regulation of the expression of these genes must be controlled, at least in part, by genes outside of the region coding for them. Furthermore, since Bosma and Owen (1977) have shown that 1 b levels are also low in C.B mice (which possess the same heavy-chain constant and variable-region genes as C57BL/10), the regulatory gene(s) must be outside the *IgH* region entirely.

The action of this regulatory gene(s) is largely specific for allotype. This is demonstrated in a comparison of 1a and 1b levels in heterozygous (BAB/14 × BALB/c) F_1 mice (see Fig. 2). In this case, the levels of 1 b are significantly lower than the levels of 1 a in the same mice.

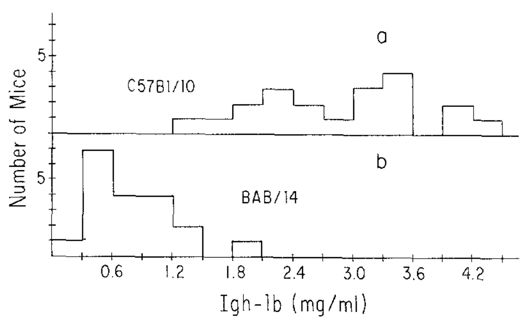

Fig. 1 a and b. Levels of Igh-1 b in the sera of C57BL/10 (**a**) and BAB/14 (**b**) mice. The probability that the two distributions are homogeneous with each other is $P<0.01$.

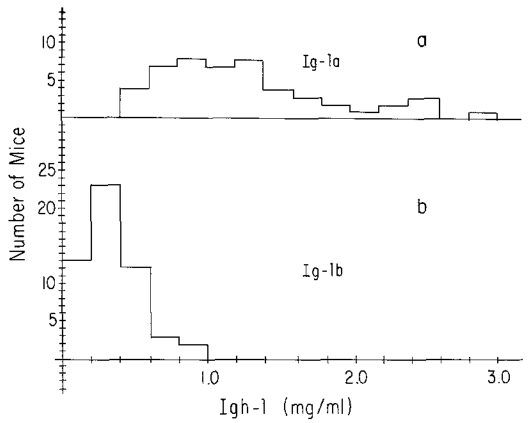

Fig. 2 a and b. Levels of Igh-1a (**a**) and Igh-1b (**b**) in the sera of (BAB/14 × BALB/c)F_1 mice.

In order to determine the location(s) and nature of the regulatory genes involved, a series of crosses was established. If the regulation observed was caused by a single dominant allele from the BALB/c background, then (C57BL/10 × BALB/c) F_1 mice would have levels as low as those of (BAB/14 × BALB/c)F_1 mice, and well below half the levels of their C57BL/10 homozygous parents. This is clearly not the case (see Fig. 3 a and b).

If a recessive allele at a single locus is responsible for the regulation observed, then one would expect that in a backcross of [(C57BL/10 × BALB/c) × BALB/c] half of the progeny would be homozygous for the regulator and half would be heterozygous. When all of the "a" allotype homozygous progeny are discarded this

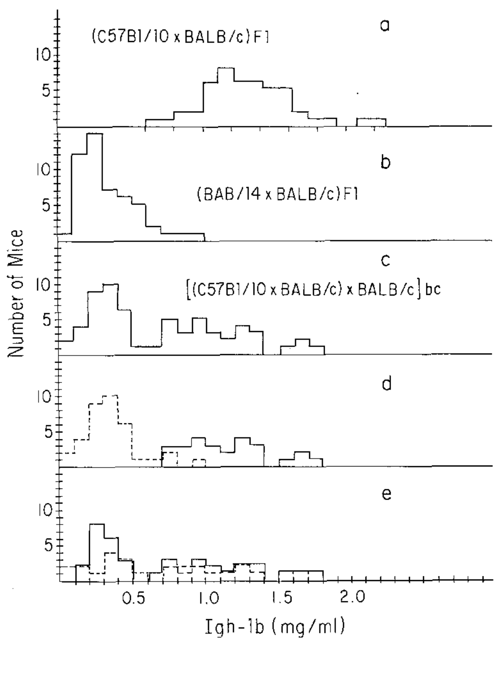

Fig. 3 a–e. Levels of Igh-1b in the sera of (C57BL/10 × BALB/c)F_1 (**a**), (BAB/14 × BALB/c)F_1 (**b**), and [(C57BL/10 × BALB/c) × BALB/c)] backcross (**c**), (**d**), (**e**) mice. Igh^a homozygous mice from the backcross are not included. In (**d**), *H-2*^{d}/*H-2*^{d} mice are represented by the *dotted line* and *H-2*^{b}/*H-2*^{d} by the *solid line*. In (**e**), albino mice are represented by the *dotted line* and nonalbino mice are represented by the *solid line*. The probability that the two distributions in (**d**) are homogeneous with each other is P <0.01.

would result in a bimodal distribution of 1b levels, one peak of the distribution being of the low (BAB/14 × BALB/c) F_1 level and one peak being of the high (C57BL/10 × BALB/c) F_1 level. Such a bimodal distribution was observed (see Fig. 3c) indicating that a single recessive allele (or closely linked set of alleles) is responsible.

In order to map the gene responsible for the suppression of 1b levels, the backcross mice from Figure 3c were typed for sex (not shown), albinism (see Fig. 3e), and *H-2* haplotype (see Fig. 3d). While no relationship was found between sex and 1b levels or between albinism and 1b levels, the $H\text{-}2^b/H\text{-}2^d$ heterozygotes had high levels of 1b and the $H\text{-}2^d/H\text{-}2^d$ homozygotes had low levels. Thus, we conclude that a locus associated with the $H\text{-}2^d$ haplotype (the BALB/c haplotype) plays a role in decreasing serum 1b levels in BAB/14 and (BAB/14 × BALB/c) F_1 mice. We have designated this locus *rig-1* and, consistent with *H-2* nomenclature, have designated the alleles as $rig\text{-}1^b$ and $rig\text{-}1^d$, in accord with their respective *H-2* haplotypes.

Further analyses of these backcross mice revealed that *rig-1* has no observable effect on Igh-4a or Igh-4b levels (see Fig. 4). However, careful analysis reveals that there is apparently a slight effect of *rig-1* on Igh-1a (1a) levels. This is revealed by the fact that 1a levels are slightly higher in (C57BL/10 × BALB/C) F_1 (rig^b/rig^d) mice than in (BAB/14 × BALB/c) F_1 (rig^d/rig^d) mice (see Fig. 5a and b), and that in the [(C57BL/10 × BALB/c) × BALB/c)] backcross this difference apparently segregates with *H-2* haplotype (see Fig. 5c and d). The effect of *rig-1* on 1a is, however, quite small compared to its effect on 1b.

To determine the location of *rig-1* with relation to the fine structure of *H-2*, we examined the progeny of crosses between BALB/c and C57BL/10 congenic mice carrying recombinant *H-2* haplotypes. First, as a control, we examined a cross between B10.D2 and BALB/c. B10.D2 mice are congenic to C57BL/10 but carry the $H\text{-}2^d$ haplotype and, therefore, presumably the $rig\text{-}1^d$ allele (the low allele). In accord with expectations, (B10.D2 × BALB/c) F_1 mice have low levels like (BAB/14 × BALB/c) F_1 mice (see Fig. 6a-c). Crosses between B10.A(3R) and BALB/c give mice that are high (see Fig. 6d). Since B10.A(3R) mice are $H\text{-}2^d$ in the *C*, *S*, and *D* subregions of *H-2*, *rig-1* must not be in this area. Therefore, we conclude that *rig-1* is somewhere to the left of the *I-C* subregion of the *H-2* chromosome region.

B10.A(3R) and B10.A(5R) differ only in the *I-J* subregion of *H-2* [B10.A(3R) is $I\text{-}J^b$, B10.A(5R) is $I\text{-}J^k$]; therefore, a difference in serum 1b levels between progeny of BALB/c and these strains would map the Ig-controlling locus to *I-J*. Among

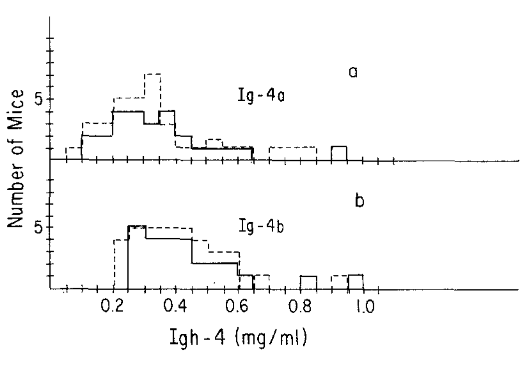

Fig. 4a and b. Levels of Igh-4a (**a**) and Igh-4b (**b**) in the sera of [(C57BL/10 × BALB/c) × BALB/C)] backcross mice. $H\text{-}2^d/H\text{-}2^d$ mice are represented by the *dotted lines* and $H\text{-}2^b/H\text{-}2^d$ by the *solid lines*. Ig^a homozygous mice from the backcross are not included.

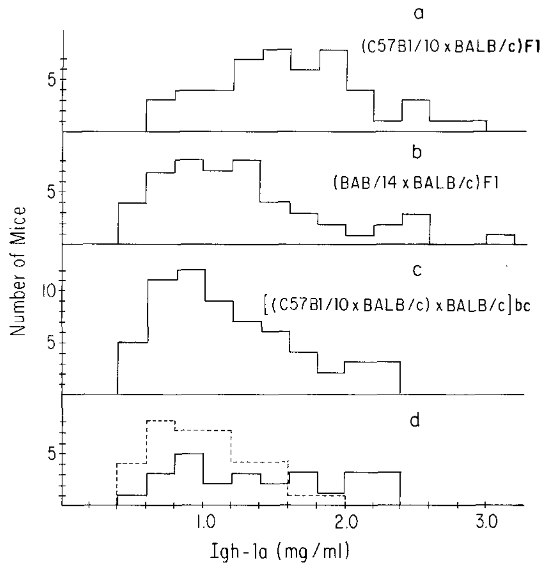

Fig. 5 a–d. Levels of Igh-1a in the sera of (C57BL/10 × BALB/c)F_1 (**a**), (BAB/14 × BALB/c)F_1 (**b**), and [(C57BL/10 × BALB/c) × BALB/c) backcross mice. In (**d**), *H-2*^{d}/*H-2*^{d} mice are represented by the *dotted line*, *H-2*^{b}/*H-2*^{d} by the *solid line*. Igh^a homozygous mice from the backcross are not included. The probability that the two distributions in (**d**) are homogeneous with each other is P = 0.02.

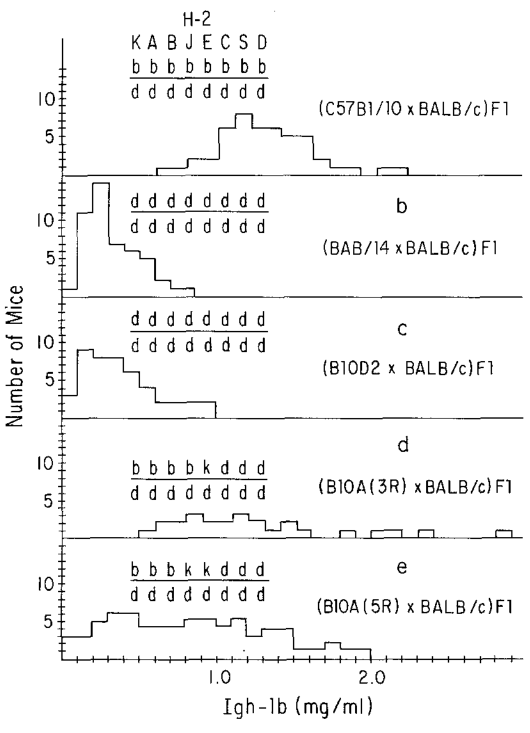

Fig. 6 a–e. Igh-1 b levels in the sera of (C57BL/10 × BALB/c)F_1 (**a**), (BAB/14 × BALB/c)F_1 (**b**), (B10.D2 × BALB/c)F_1 (**c**), (B10.A(3R) × BALB/c)F_1 (**d**), and (B10.A(5R) × BALB/c)F_1 (**e**) mice. *H-2* genotypes are included. The probability that the distributions in (**c**) and (**d**) are homogeneous with each other is P < 0.01. The probability that the two distributions in (**d**) and (**e**) are homogeneous with each other is P = 0.025.

progeny derived from the B10.A(5R) × BALB/c cross, we did find several animals with low levels not found in the B10.A(3R) × BALB/c cross (see Fig. 6e). However, since the distributions are equivalent, with the exception of the low animals, we believe that it would be unwise to claim that *rig-1* maps to *I-J*. A repeat of this experiment (Dr. Seiichi Kobayashi, personal communication) has not resolved this question. If *rig-1* is in *I-J*, it is likely that it will require congenic mice differing at *I-J* for the *d* and *b* alleles rather than for the *k* and *b* alleles to prove it.

Dominant BALB/c allele(s) contribute to 1b regulation. Although (B10.D2 × BALB/c) F_1 mice have low levels of 1b consistent with the fact that they are *rig-1*^{d}/*rig-1*^{d}, we have not been able to find any significant difference between the levels of 1b in the B10.D2 and C57BL/10 strains themselves. This indicates that the rig-1^d/rig-1^d phenotype is not sufficient to cause a lowering of 1b levels without the BALB/c background being present. This implies that some gene or genes from the BALB/c background (which we can provisionally designate as *Rig-2*) must act in concert with *rig-1* for regulation to occur. Since *Rig-2* must act in the heterozygous (B10.D2 × BALB/c) F_1 mice, its BALB/c allele (*Rig-2*^{a}) must be dominant over the B10 allele (*Rig-2*^{b}).

Discussion

We have presented evidence that at least two unlinked loci are involved in the regulation of the production of immunoglobulin by Igh-1b. In a separate publication we will present evidence that this regulation occurs in the form of T-cell-mediated, allotype-specific suppression. However, this is not the full story of the genetic control of allotype suppression in mice. Other alleles and other loci might be involved.

From previous work, we know that B10.S (a C57BL/10 congenic line carrying the *H-2*^{s} haplotype) mice mated to BALB/c females produce progeny with normal (high) levels of 1b. However, when B10.S mice are mated to BALB/c females immunized against "b" allotype immunoglobulins, they give progeny with low levels of 1b (Herzenberg and Herzenberg 1974). Since such a dichotomy does not exist when C57BL/10 mice are used as the fathers (L. A. Herzenberg, unpublished data), it is clear that the *rig-1*^{s} allele must differ from both *rig-1*^{b} and *rig-1*^{d}. Furthermore, from the data we have presented showing a possible difference between (B10.A(3R) × BALB/c) F_1 mice and (B10.A(5R) × BALB/c) F_1 mice, it is also possible that *rig-1*^{k} is different from *d*, *s*, and *b*.

The (B10.S × im BALB) cross also raises another important point. We know that SJL mice crossed with BALB/c females immunized against "b" allotype immunoglobulins produce progeny with a degree of suppression far greater than that observed when B10.S mice are the fathers. Since (B10.S × imBALB/c) F_1 mice and (SJL × imBALB/c) F_1 mice are both *rig-1*^{s}/*rig-1*^{d} and heterozygous for the BALB/c allele at *Rig-2*, then some other allele or locus must be responsible for the difference. Whether it is simply another allele at *Rig-2* from SJL that is responsible or whether it is another locus (*Rig-3*?) or set of loci is not possible to define with current data. *Rig-2* itself has not been defined as a single locus, so the precise number of loci and alleles controlling allotype suppression in mice must remain a mystery.

The best defined controlling locus is *rig-1*. Its definition as a single locus suffers only from the normal drawbacks associated with defining eukaryotic loci, that is, that it may be two or more closely linked loci. The fine mapping of *rig-1* is, however, somewhat more equivocal. We feel confident from the data presented that *rig-1* maps to the left of the *I-C* subregion. However, we do not feel the data implying its location in *I-J* are conclusive, and we present them only for informational value not for the pupose of drawing conclusions. If *rig-1* is located in *I-J*, it would be especially interesting since we already know that the *I-J* region codes for an antigen on suppressor T cells (Murphy et al. 1976).

Finally, we would like to note a relationship between genes and function. Genes do not function in a vacuum. Each allele must have an effect on some subpopulation of cells possessing it. Whether an allele produces a variant of an antigen or receptor molecule, or whether it produces a promotor or repressor which interacts with DNA at affected loci is not known for this system. But each locus and allele defined in this study should have a definable cellular effect on the process of allotype suppression. It is hoped future studies will define these interactions.

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