# Toward genetic dissection of high and low antibody responsiveness in Biozzi mice

(mapping/quantitative trait loci/immunomodulatory genes/backcrosses)

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**ABSTRACT** Several distinct chromosomal segments were recently identified by cosegregation analysis of polymorphic markers with antibody responsiveness in an $F_2$ cross between high (H) and low (L) antibody responder lines of Biozzi mice. The effect associated with the relevant markers has now been investigated in backcross populations (toward the L line) bred from H and L mice made coisogenic at the *H-2* locus. The antibody titers, measured on days 5 and 14 of the primary response to sheep red blood cells, were considered to be two distinct quantitative phenotypes. The results of single or multilocus analyses demonstrated the significant involvement, at one or the two titration times, of *Im* gene(s) on four distinct chromosomes: 4, 8, 12, and 18. The regions on chromosomes 6 and 10 have a lesser but still suggestive effect. The contribution of each locus ranged from 3% to 13%, and together these loci accounted for about 40% of the phenotypic variance at each titration time. The data are compatible with an additive effect of the relevant loci and suggestive of some interaction effects. In a second backcross toward L line, the H line alleles of the putative *Im* genes on chromosomes 6, 8, and 12 were isolated from each other and their effects were still detected.

Quantitative antibody (Ab) responsiveness to natural immunogens is a multifactorial trait characterized by a continuous variability among genetically heterogeneous populations. Many experiments, carried out in inbred strains of mice, analyzing the role of candidate genes on Ab responsiveness (principally the *H-2* and *Igh* loci) have also provided evidence for large background effects (1–4). The production of high (H) and low (L) Ab responder lines of mice by selective breeding for Ab production to heterologous erythrocytes has opened the way to investigate the multigenic control of quantitative Ab responses (5). It was estimated that 10 independently segregating loci endowed with additive effects are responsible for the huge (240-fold) multispecific difference separating H and L Ab titer phenotypes (6). Recently, a genome-wide search for the mapping of the relevant genes was undertaken; the genotyping for 90 informative microsatellite markers was restricted to the mice giving the highest and lowest Ab responses among a large immunized $(H \times L)F_2$ population (7). The frequency deviation of parental genotypes (measured by a $\chi^2$ test) in the two $F_2$ groups clearly indicated the presence, in close vicinity to several markers, of gene(s) contributing to the H/L difference. This screening confirmed the known involvement of *Igh*- (8) and *MHC*- (9, 10) linked genes, located on chromosomes 12 and 17, respectively ($P < 10^{-5}$ for *D12Nds2* and $10^{-4}$ for *D17Mit13* markers), and demonstrated the presence of an unsuspected immunomodulatory (*Im*) gene on chromosome 6 ($P = 2.10^{-4}$ for the *D6Mit5* marker). In addition, the presence of *Im* genes could be considered on five distinct chromosomes, as corresponding $P$ values were around $10^{-2}$ for *D2Mit9*, *D4Mit31*, *D8Mit35*, *D10Mit14*, and *D18Mit19* markers on chromosomes 2, 4, 8, 10, and 18, respectively (7).

The next steps consisted of establishing the possible involvement of *Im* genes on chromosomes 2, 4, 8, 10, and 18 and measuring the effect of *Im* gene(s) at each region [quantitative trait loci (QTL) analysis] in a whole hybrid population (11). Such an evaluation of the QTL effect on parameters with continuous variation has already been reported in several animal models (12–17). We chose to study a backcross to L line because of the partial dominance of high-over-low anti-sheep red blood cell (SRBC) responses (6), with the risk, however, of an impaired detection of any gene having an opposite dominance direction.

The present results establish for the first time, to our knowledge, the involvement of three new *Im* genes on chromosomes 4, 8, and 18, provide an approximative localization of all the *Im* genes detected so far in the H and L lines, and point to their relative contribution and possible interactions.

## MATERIALS AND METHODS

**Mice and Crosses.** H and L mice and their crosses were bred in our animal facility under specific pathogen-free conditions (Institut Curie).

The H progenitors of $F_1$ hybrids were derived from the H subline made coisogenic for the L line H-2 haplotype (H-2ˢ), by 12 consecutive backcrosses.

The $(H \times L)F_1 \times L$ backcross population resulted from two successive mating series (BcL1a and BcL1b), as only half of the first pairs were productive. BcL1a and BcL1b groups consisted of 64 and 47 mice, respectively.

The second backcross (BcL2) population of 212 mice was generated from BcL1 parents selected for their genotype at various microsatellites.

**Phenotype Measurement.** Mice received a single intravenous injection of $5.10^8$ SRBC per mouse. Individual blood samples were collected on days 5 and 14 after immunization. Sera, kept frozen, were simultaneously titrated.

The BcL1a and BcL1b mice were immunized when they were 2 months old using SRBC from the same donor. Though the genotype distribution at each locus was similar in the two series, the mean Ab responses to SRBC differed: the agglutinin titers ($\log_2$) were $7.5 \pm 1.1$ and $5.2 \pm 1.4$ (on day 5) and $7.8 \pm 1.1$ and $5.2 \pm 1.8$ (on day 14) in BcL1a and in BcL1b series, respectively. On the presumption that this was due to SRBC

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Abbreviations: Ab, antibody; H mice, high antibody producer mice; *h*, H line allele; L mice, low antibody producer mice; *l*, L line allele; BcL, $(F_1 \times L)$ backcross; SRBC, sheep red blood cells; *Im* genes, immunomodulatory genes; QTL, quantitative trait loci; LRS, likelihood ratio statistic; cM, centimorgan.
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batch, the male mice were subsequently immunized by two i.p. injections of killed *Salmonella typhimurium* at 3-week intervals. No difference was observed between the two series in anti-flagellar Ab titers, measured by an ELISA assay 2 weeks after the second injection (18) (data not shown). The difference in SRBC Ab responses between BcL1a and BcL1b series was thus attributable to an environmental factor, and the statistical analyses were done on grouped data using a standardized variate, to ensure an unimodal distribution, as follows: $(x - m)/s$, where x is the individual Ab titer, m is the mean Ab titer of the population (males and females, separately), and s is the corresponding standard deviation. Tests of homogeneity (19) were performed from the mean Ab titers calculated separately in the two series for heterozygous (*hl*) and homozygous (*ll*) individuals at each marker to assess the risk of a biased measurement of gene effect. These tests were satisfactory, meaning a concordant gene effect in the two series, for all markers except for *D2Mit9* on chromosome 2, for which grouped data were not used. The consistency of the results obtained from grouped data (see Tables 1–3) with those calculated in the two BcL1 series separately was verified for all the statistical tests.

**DNA Extraction.** Mouse DNA was extracted from a frozen small tail segment. The ground sample was incubated at 65°C in 100 $\mu$l of lysis buffer containing 50 mM Tris·HCl (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS, and 1.5 mg/ml proteinase K (Boehringer Mannheim) for 1 hr; 100 $\mu$l of the same buffer without proteinase K was added for 15 min. The samples were centrifuged (11,000 rpm for 10 min), and the supernatants were mixed with 0.1 vol of 3 M sodium acetate (pH 5.2) and 2 vol of 100% ethanol. The DNA precipitates were washed, briefly air-dried at room temperature, and dissolved in sterile water (T. Roger, personal communication).

**Microsatellite Genotyping.** The microsatellite primers (20) were purchased from Research Genetics (Huntsville, AL). The following microsatellites were used: *D1Mit14* [on chromosome 1 at 82 centimorgans (cM) from the centromere]; *D2Mit9* (on chromosome 2 at 37 cM), *D3Mit14* (on chromosome 3 at 64 cM); *D4Mit5*, *D4Mit31*, and *D4Mit40* (on chromosome 4 at 17, 44, and 54 cM, respectively); *D6Mit33*, *D6Mit16*, *D6Mit5*, *D6Mit10*, and *D6Mit15* (on chromosome 6 at 26, 32, 36, 50, and 70 cM, respectively); *D8Mit9*, *D8Mit35*, and *D8Mit42* (on chromosome 8 at 32, 62, and 69 cM, respectively); *D10Mit42* and *D10Mit14* (on chromosome 10 at 43 and 63 cM, respectively); *D12Mit9*, *D12Mit33*, *D12Mit27*, and *D12Nds2* (on chromosome 12 at 10, 29, 49, and 50 cM, respectively); *D18Mit19*, *D18Mit12*, and *D18Mit8* (on chromosome 18 at 2, 12, and 43 cM, respectively). The distances (from the centromere) are those calculated from the recombination ($\theta$) events calculated from our experimental data and that fit those reported in the Mouse Genome Data Base.

PCR genotyping was performed as described (21). Briefly, amplification of DNA was performed in a Techne Thermal Cycler PHC3 (Techne Laboratories, Princeton), in a final volume of 10 $\mu$l in a 96-well U-bottom microtiter plate (Techne Laboratories) using the following program: 3 min at 94°C, 35 cycles of 50 sec at 94°C, 1 min at 55°C, and 1 min at 72°C followed by a final extension step of 3 min at 72°C. After addition of 30 $\mu$l of formamide dye, the samples were denatured for 5 min at 94°C and 2–4 $\mu$l were electrophoresed in 6% denaturing acrylamide gels for 2–3 hr at 50 W. Gels were then autoradiographed without drying for 4 to 24 hr at −80°C.

**Statistics.** The quantitative effect of each locus was measured by the difference between the mean Ab responses of heterozygous (*hl*) and homozygous (*ll*) individuals, and the significance was tested using a Student's *t* test.

At each marker, the presence of a QTL was tested by simple regression analysis using the program MAPMANAGER QTB6 (a program for genetic mapping released in April 1996 by K. Manly and R. Cudmore, Roswell Park Cancer Institute).

Simple and composite interval mapping algorithms (simple or multiple regression), implemented in the same software package, were also used. In composite interval mapping analyses, the likelihood ratio statistic (LRS) on each chromosome was estimated with the most significant marker of all the other chromosomal segments as controlling the effects of background (22–25). For each chromosome, three significance threshold values, intrinsic to the experimental data set, were calculated (26). They correspond to values at the 75.0th, 97.0th, and 99.9th percentiles of a LRS distribution derived from 2000 random permutations of the phenotypes relative to the genotypes. These three values are considered as "suggestive," "significant," and "highly significant" thresholds. Reproducible values were obtained in duplicate permutation tests. For each chromosome, the three threshold values shown in Table 2 were calculated under the same conditions as those used for the LRS calculation.

For each LRS peak, a QTL position interval was calculated on the chromosome map as the 100:1 odds region, in which LRS = $\ln(H1/H0) > \text{LRS(QTL)} - \ln(100)$, where H0 and H1 are the null and "QTL is present" hypotheses, respectively, and LRS(QTL) is the LRS at the peak.

Analysis of variance was performed using 4V program in the BMDP statistical software package (BMDP Statistical Software, Los Angeles, CA). Calculations were made taking into account six factors (represented by the most significant markers on each chromosome) under a monofactorial or multifactorial model. In the latter, the effects of each factor were evaluated either assuming no interaction or taking into account 2-by-2 factor interactions. More complex interactions were not tested due to population size.

## RESULTS

**Quantitative Effect of *Im* Loci in (H×L)F$_1$ × L Backcross.** The increase of antibody titer associated with the presence of H line allele at one or several adjacent polymorphic markers defines a QTL. This effect was investigated in a backcross population (BcL1) at all the markers suspected to reveal a QTL on the basis of our previous screening carried out in two selected groups of a (H×L)F$_2$ cross. As the kinetics of Ab responses differ in H and L lines of mice, QTL effect was calculated separately on days 5 and 14 of the primary response to SRBC.

Table 1 shows the mean standardized Ab titers in BcL1 mice with homozygous (*ll*) or heterozygous (*hl*) genotype at each marker. The difference between these two values measures the quantitative effect of putative *Im* gene(s). For simplicity, at each region the result of the marker yielding the most significant difference in day 5 titers is indicated. The same markers also gave maximal scores on day 14, except for chromosomes 4 and 12 where *D4Mit31* and *D12Mit33* gave more significant *P* values (0.0016 and 0.0180, respectively).

These results strongly support that all the regions suggested by our previous F$_2$ screening contain QTL(s) with immunomodulatory effect, except for the chromosome 2 region identified by the *D2Mit9* marker. Nevertheless, a significant effect at this marker (*hl-ll* difference being 1.2, $P = 0.002$) was observed in the BcL1b series (see *Materials and Methods*).

Most QTL effects varied when measured on the early or late stage of the response, the greatest difference between the two titrations being observed for the *D12Nds2* marker. The strongest *Im* gene effects in terms of *hl-ll* difference are associated with the *D4Mit5* and *D12Nds2* markers on day 5 and with the *D8Mit35* marker on day 14 after immunization.

To ascertain the statistical significance of QTL(s) detection, we relied on significance thresholds, intrinsic to our experimental data, derived from permutation tests using the MAP-MANAGER package software (see *Materials and Methods*). Table 2 shows the maximal LRS scores obtained under an

Table 1.    Quantitative effect of *Im* genes on Ab responses to SRBC in BcL1

| Chromosome | Markers | cM | Day 5 Ab titers | | | Day 14 Ab titers | | |
|---|---|---|---|---|---|---|---|---|
| | | | *ll*\* | *hl*\* | *P(hl − ll)* | *ll*\* | *hl*\* | *P(hl − ll)* |
| 4 | *D4Mit5* | 17 | −0.29 ± 0.12 | 0.37 ± 0.14 | $3.0 \times 10^{-4}$ | −0.23 ± 0.12 | 0.29 ± 0.14 | $5.1 \times 10^{-3}$ |
| 6 | *D6Mit5* | 36 | −0.25 ± 0.12 | 0.28 ± 0.14 | $4.6 \times 10^{-3}$ | −0.22 ± 0.11 | 0.25 ± 0.15 | $1.3 \times 10^{-2}$ |
| 8 | *D8Mit35* | 62 | −0.22 ± 0.12 | 0.26 ± 0.13 | $1.0 \times 10^{-2}$ | −0.29 ± 0.12 | 0.33 ± 0.13 | $9.0 \times 10^{-4}$ |
| 10 | *D10Mit14* | 63 | −0.19 ± 0.12 | 0.30 ± 0.13 | $1.1 \times 10^{-2}$ | −0.14 ± 0.12 | 0.22 ± 0.15 | $5.8 \times 10^{-2}$ |
| 12 | *D12Nds2* | 50 | −0.33 ± 0.14 | 0.31 ± 0.12 | $5.0 \times 10^{-4}$ | −0.09 ± 0.13 | 0.09 ± 0.14 | $3.4 \times 10^{-1}$ |
| 18 | *D18Mit12* | 12 | −0.26 ± 0.14 | 0.21 ± 0.12 | $1.3 \times 10^{-2}$ | −0.31 ± 0.14 | 0.24 ± 0.12 | $3.4 \times 10^{-3}$ |

Standardized Ab titers values are mean ± SEM.
\*Mouse genotypes.

interval mapping strategy, together with the corresponding significance thresholds. Under simple interval mapping strategy (simple regression model), QTLs on chromosomes 4 and 12 (on day 5) and 4 and 8 (on day 14) reached scores within the range of significant to highly significant threshold values, while the other QTL scores ranged between suggestive and significant values.

However, in the simple regression model, the presence of several QTLs is likely to interfere. We therefore resorted to composite interval mapping strategy for a more reliable estimate of the LRS score and position of each QTL. *Im* genes on chromosomes 4, 8, 12, and 18 then reached scores ranging from significant to highly significant or even higher, whereas scores of QTL(s) on chromosomes 6 and 10 were not improved. The QTL position deduced from the two mapping strategies were remarkably stable, except that of QTLs on chromosomes 4 and 6. The 100:1 odds intervals of placing the QTLs are comprised within 22 to 40 cM interval.

The relative effect of each locus was estimated by analysis of variance under models of increasing complexity: (*i*) monofactorial and (*ii*) multifactorial considering no or 2-by-2 factor interactions between involved loci (Table 3). The comparison of the results obtained under either monofactorial (corresponding to the *t* test results shown in Table 1) or multifactorial models exhibits sensible differences, namely, a decrease of *D6Mit5*- and *D10Mit14*-associated effects and an increase of *D12Nds2*- and *D18Mit12*-associated effects. Under the multifactorial model, most QTL effects remained constant whether or not 2-by-2 interaction variance was taken into account. However, in some of the locus pairs tested, interaction variance estimates attained significant values, namely *D4Mit31*–*D6Mit5* ($P = 10^{-2}$), *D4Mit31*–*D18Mit12* ($P = 4.10^{-2}$), and *D6Mit5*–*12Nds2* ($P = 4.10^{-2}$) on day 5, and *D4Mit5*–*D12Mit33* ($P = 10^{-2}$), *D6Mit5*–*D18Mit12* ($P = 2.10^{-2}$), and *D6Mit5*–*D12Nds2* ($P = 6.10^{-4}$) on day 14 (data not shown).

Altogether the detected loci explained 38% and 43% of the total variance on day 5 under no and 2-by-2 interaction models,

respectively, and 40% of the total variance on day 14 for both calculations (data not shown).

**Toward the Isolation of the Distinct QTLs.** Further backcrosses to L mice were bred to produce "fast congenic" lines of mice for H line alleles at the identified loci in an attempt to define their respective effect. The parents were therefore selected according to their genotype to accelerate the separation of the distinct QTLs. For the second backcross (BcL2), the BcL1 parents were chosen, based on the $F_2$ cross results, to avoid firstly coinheritance of the putative *Im* genes on chromosomes 6, 8, and 12. Three populations (A, B, and C) were produced, each one from BcL1 males heterozygous at only one of the three loci located on chromosome 6, 8, or 12, respectively. The frequency of the selected marker was therefore approximately 25% and that of H background was 12.5%. Heterozygosity of the parents at the other QTLs (on chromosomes 4, 10, and 18) was uncontrolled and varied in the three populations. The results for these "unselected" loci are presented in Table 4 separately for the three populations, because of gene frequency differences and only when more than 10 mice were heterozygous. The mean antibody titers were similar in the three populations, ranging between 6.52 and 6.98 $\log_2$ on day 5 and 5.92 and 6.17 $\log_2$ on day 14. The QTL effects were only estimated from differences between mean values in *ll* and *hl* individuals and are given for the titration time showing the most significant effect (generally on day 14 of the response).

As shown in Table 4, the presence of one h allele at the QTLs of chromosomes 6, 8, or 12 separately was still able to increase antibody responsiveness, although with less significant values than those indicated in Table 1. The effect of chromosome 4 QTL in groups A and B (but not in group C) and of chromosome 18 QTL in groups A and C remained strong (Table 4), whereas the chromosome 10 QTL effect was undetectable in groups B and C (data not shown).

## DISCUSSION

The present article describes a QTL analysis of six chromosomal segments on chromosomes 4, 6, 8, 10, 12, and 18, two of

Table 2.    QTL interval mapping in BcL1 population

| Titration time | Chromosome | Simple regression analysis | | | | | Multiple regression analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cM | Peak LRS | Sug | S | HS | cM | Peak LRS | Sug | S | HS |
| Day 5 | 4 | 30 | 15.4 | 7.6 | 12.0 | 15.4 | 45 | 15.1 | 8.0 | 12.7 | 19.8 |
| | 6 | 46 | 9.8 | 7.7 | 12.0 | 19.0 | 37 | 8.1 | 7.9 | 12.3 | 17.8 |
| | 8 | 60 | 8.7 | 7.6 | 11.5 | 18.6 | 64 | 9.0 | 7.9 | 12.5 | 20.0 |
| | 10 | 63 | 6.7 | 6.6 | 10.7 | 15.6 | 63 | 5.8 | 7.2 | 11.2 | 18.4 |
| | 12 | 47 | 11.1 | 6.9 | 10.9 | 18.8 | 44 | 21.0 | 8.0 | 12.7 | 19.2 |
| | 18 | 11 | 6.3 | 6.0 | 12.5 | 19.6 | 11 | 13.5 | 8.2 | 12.4 | 21.5 |
| Day 14 | 4 | 40 | 12.8 | 7.7 | 11.8 | 20.0 | 57 | 17.1 | 7.9 | 12.6 | 20.7 |
| | 6 | 39 | 6.4 | 7.5 | 12.1 | 18.7 | 28 | 7.3 | 8.0 | 12.8 | 18.4 |
| | 8 | 63 | 13.4 | 7.4 | 11.5 | 18.5 | 67 | 15.0 | 8.2 | 12.9 | 18.8 |
| | 10 | 63 | 3.7 | 6.7 | 10.5 | 17.3 | 63 | 3.5 | 7.3 | 11.6 | 15.0 |
| | 12 | 29 | 5.3 | 7.7 | 12.3 | 17.8 | 29 | 13.8 | 6.8 | 11.6 | 16.0 |
| | 18 | 11 | 8.6 | 6.9 | 10.5 | 16.4 | 11 | 19.1 | 5.0 | 9.1 | 13.1 |

Significance threshold values: Sug, suggestive; S, significant; HS, highly significant.

Table 3. Analysis of variance in BcL1 population

| Titration time | Markers | Monofactorial model | | | Multifactorial model | | | | | |
| | | | | | No interaction | | | 2-by-2 factor interactions | | |
| | | R.E.*, % | F | P values | R.E.*, % | F | P values | R.E.*, % | F | P values |
|---|---|---|---|---|---|---|---|---|---|---|
| Day 5 | D4Mit31 | 7.7 | 8.2 | $5.1 \times 10^{-3}$ | 8.3 | 13.5 | $4.0 \times 10^{-4}$ | 9.7 | 16.1 | $1.0 \times 10^{-4}$ |
| | D6Mit5 | 7.9 | 8.4 | $4.6 \times 10^{-3}$ | 4.7 | 7.6 | $6.9 \times 10^{-3}$ | 3.8 | 6.3 | $1.4 \times 10^{-2}$ |
| | D8Mit35 | 6.5 | 6.8 | $1.0 \times 10^{-2}$ | 4.0 | 6.5 | $1.2 \times 10^{-2}$ | 3.8 | 6.3 | $1.4 \times 10^{-2}$ |
| | D10Mit14 | 6.4 | 6.8 | $1.1 \times 10^{-2}$ | 3.2 | 5.3 | $2.4 \times 10^{-2}$ | 3.0 | 4.9 | $2.9 \times 10^{-2}$ |
| | D12Nds2 | 11.7 | 13.0 | $5.0 \times 10^{-4}$ | 13.2 | 21.4 | $<1.0 \times 10^{-4}$ | 15.0 | 24.9 | $<1.0 \times 10^{-4}$ |
| | D18Mit12 | 6.0 | 6.3 | $1.3 \times 10^{-2}$ | 8.6 | 14.0 | $3.0 \times 10^{-4}$ | 10.6 | 17.7 | $1.0 \times 10^{-4}$ |
| Day 14 | D4Mit31 | 9.7 | 10.5 | $1.6 \times 10^{-3}$ | 10.4 | 16.7 | $1.0 \times 10^{-4}$ | 9.2 | 14.7 | $2.0 \times 10^{-4}$ |
| | D6Mit5 | 6.1 | 6.4 | $1.3 \times 10^{-2}$ | 3.2 | 5.1 | $2.6 \times 10^{-2}$ | 4.0 | 6.4 | $1.3 \times 10^{-2}$ |
| | D8Mit35 | 10.7 | 11.8 | $9.0 \times 10^{-4}$ | 8.2 | 13.2 | $4.0 \times 10^{-4}$ | 9.4 | 14.9 | $2.0 \times 10^{-4}$ |
| | D10Mit14 | 3.6 | 3.7 | $5.8 \times 10^{-2}$ | 1.9 | 3.1 | $8.3 \times 10^{-2}$ | 0.8 | 1.2 | $3.0 \times 10^{-1}$ |
| | D12Mit33 | 5.5 | 5.8 | $1.8 \times 10^{-2}$ | 9.4 | 15.1 | $2.0 \times 10^{-4}$ | 9.0 | 14.3 | $3.0 \times 10^{-4}$ |
| | D18Mit12 | 8.3 | 9.0 | $3.4 \times 10^{-3}$ | 12.6 | 20.3 | $<1.0 \times 10^{-4}$ | 12.1 | 19.3 | $<1.0 \times 10^{-4}$ |

*Relative effect: R.E., % = (mean square/total square sum) × 100.

them already known (on chromomes 6 and 12) to contain *Im* genes contributing to the H and L antibody responder phenotypes (Biozzi mice) (7). The *Im* gene effect was measured in a (H×L)F$_1$ × L backcross in terms of Ab production increase associated with H line alleles, at the markers selected from a previous (H×L)F$_2$ screening (7).

Ab titers were measured on days 5 and 14 postimmunization, as maximal Ab titers are reached on day 5 (IgM response) in L mice, and on day 14 (IgG response) in H mice (27). These two parameters were used separately in all analyses, as gene effect might differ at the two stages of antibody responses (28). Indeed, in BcL1, the effect and/or position of QTLs differed according to titration times (Tables 1–3), suggesting either the involvement of distinct genes in the same chromosomal segment (as suspected for chromosomes 4 and 12) or distinct gene interactions. Kinetic differences were also observed in BcL2, as most loci, except chromosome 18 locus, preferentially affected the 14th day response.

In BcL1, the *P* values of QTL effect on chromosomes 4, 8, and 12 were close to the $10^{-4}$ value required for formal linkage demonstration (29), while the significance at the three other regions (chromosomes 6, 10, and 18) was < 0.01 (Table 1).

Among the regions detected with low $\chi^2$ scores on the F$_2$ analysis, only the region on chromosome 2 still gave questionable results. In fact, the *D2Mit9*-associated effect might be detectable only on L Ab responder phenotype as it was significant only in the BcL1b series.

Table 4. Quantitative effect of *Im* genes on Ab responses to SRBC in three distinct BcL2 populations

| BcL2 populations | Markers | Day 14 Ab titers | | |
| | | ll* | hl* | P(hl − ll) |
|---|---|---|---|---|
| A | | | | |
| (n = 63[†]) | D6Mit5 | −0.20 ± 0.17 | 0.41 ± 0.21 | $2.5 \times 10^{-2}$ |
| | D4Mit40 | −0.13 ± 0.16 | 0.77 ± 0.16 | $4.1 \times 10^{-3}$ |
| | D18Mit19[‡] | −0.13 ± 0.18 | 0.63 ± 0.16 | $4.7 \times 10^{-3}$ |
| B | | | | |
| (n = 95[†]) | D8Mit9 | −0.34 ± 0.22 | 0.38 ± 0.14 | $5.8 \times 10^{-3}$ |
| | D4Mit40 | −0.17 ± 0.22 | 0.33 ± 0.15 | $5.5 \times 10^{-2}$ |
| C | | | | |
| (n = 54[†]) | D12Nds2 | −0.35 ± 0.22 | 0.20 ± 0.16 | $4.5 \times 10^{-2}$ |
| | D4Mit40 | 0.05 ± 0.18 | −0.11 ± 0.19 | $5.7 \times 10^{-1}$ |
| | D18Mit19[‡] | −0.11 ± 0.13 | 1.11 ± 0.18 | $1.0 \times 10^{-4}$ |

Standardized Ab titer values are mean ± SEM.
*Mouse genotypes.
[†]n = number of mice.
[‡]Day 5 titers.

The two regions containing the candidate Fcγ receptors coding genes (close to the *D1Mit14* and *D3Mit14* markers), which were found to be unlinked in the F$_2$ hybrid analysis, still gave no evidence of QTL effect (data not shown).

The finding of coherent results in two completely independent hybrid populations (F$_2$ and backcross) therefore strengthens the validity of *Im* gene detection. Nevertheless, the significance rank order of the distinct regions varies in the two analyses. This may be partly due to the exclusion of the *H-2* locus polymorphism, to a greater impact of the dominance effect or to a reduced *Im* gene(s) frequency in the backcross mice compared with the two extreme F$_2$ groups.

A more refined analysis was provided by simple interval mapping, which gave a fully reliable calculation of the significance thresholds (26). However, the best estimates of QTL scores and localization were obtained by the use of the composite interval mapping algorithm, in which other QTL effects are accounted for (22–25). Noticeable changes were then observed for chromosome 4 and 6 QTL positions; meanwhile, LRS scores of chromosome 12 and 18 QTLs increased up to highly significant values (Table 2).

The *F* values of the variance analysis reported in Table 3 also slightly increased for markers on chromosomes 4, 12, and 18 under multifactorial versus monofactorial models.

The finding that the six identified QTLs accounted for about 40% of the overall phenotypic variance in BcL1, without evidence for a major gene effect, fits with our previous estimate of genetic versus environmental variance partition in (H×L) segregant hybrids (6). This indicates that most of the *Im* genes contributing to H/L difference were presumably detected.

The decrease of chromosome 6 and 10 effect using a multifactorial model (Table 3) may be indicative of interactions with other *Im* genes. Indeed, the *D6Mit5* marker was frequently involved in significant interaction variances. Using a cross between lines of mice previously selected for extreme phenotypes theoretically provided a model for investigating interaction effects between QTLs. However this investigation was limited by the BcL1 population size and some imprecise QTL locations. Nevertheless, as most of QTL scores do not drastically change under the three models of variance analysis, the genes can be supposed to operate mainly through additive effects.

This supported the strategy of isolating the QTLs in fast congenic lines of mice (30). In fact, most *Im* genes still had a detectable effect in BcL2 mice (Table 4). In this cross, reduced H line background allele frequency (from 25% to 12.5%) may modify the expression or dominance of *Im* genes. In addition, the most drastic change in the BcL2 mouse population was the

decrease of *Im* gene coexpression and/or interaction, deliberately accelerated by selecting the parents on the basis of their genotype. Results on BcL2 indicate that the *Im* genes on chromosomes 6, 8, and 12 are at least partially independent of each other in terms of their phenotypic expression, as they still have an effect on Ab responses. The fact that chromosome 10 QTL was no more detected could be related to a dominance effect, as recent results demonstrated a highly significant increase of Ab responses in mice homozygous for the H line allele at the *D10Mit14* marker (unpublished data).

The use of few markers selected from our previous $F_2$ hybrid analysis allowed us to give here a first estimation of *Im* genes localization. In our conditions (map marker density and population size), the confidence intervals of QTL mapping are still large and may include several QTLs in the same segment. However, within our QTL(s) interval limit, some candidate genes can be proposed and others excluded. The more relevant candidates in the distinct QTLs are the *Ifa* and *Ifb* genes on chromosome 4; the *Tcrb*, *Igk*, *Cd8*, and *Bphs* (31) genes on chromosome 6; the genes coding for esterases on chromosome 8; the genes coding for γ interferon and the signal transducer and transcription activator Stat6 (32, 33) on chromosome 10; the *Igh* locus on chromosome 12; and the genes coding for adhesion molecules (cadherins) and peptidase on chromosome 18. However, the presence of candidate genes does not exclude that other genes might be responsible for the effect.

Detailed studies of the phenotypic expression of each locus, in fast congenic lines, will help to identify new genes and/or discriminate among the various candidates. These lines will also be useful to investigate the role of the distinct *Im* genes in the physiopathological modifications associated with H and L genotypes and particularly their opposite pattern of resistance against bacterial and parasitic infections and their differences in spontaneous tumors occurrence and life span (34).

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