A Novel Locus Regulates Both Retroviral Glycoprotein 70 and Anti-Glycoprotein 70 Antibody Production in New Zealand Mice When Crossed with BALB/c


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A Novel Locus Regulates Both Retroviral Glycoprotein 70 and Anti-Glycoprotein 70 Antibody Production in New Zealand Mice When Crossed with BALB/c1


Lupus-prone New Zealand Black and New Zealand White mice produce high serum levels of the endogenous retroviral envelope protein gp70 and develop an Ab response to this autoantigen as part of their autoimmune disease. Linkage analysis of two crosses involving New Zealand and BALB/c mice mapped these traits to a group of overlapping loci, including a novel locus on proximal chromosome 12. This locus was linked with serum gp70 and the autoimmune response against it. The linkage of serum gp70 levels to a previously described locus on distal chromosome 4 was also confirmed. Sequence analysis of a candidate gene on distal chromosome 4, Fv1, provided support that this gene may be associated with the control of serum gp70 levels in both New Zealand Black and New Zealand White mice. Linkage data and statistical analysis confirmed a close correlation between gp70 Ag and anti-gp70 Ab levels, and together gave support to the concept that a threshold level of gp70 is required for the production of anti-gp70 Abs. Serum levels of anti-gp70 Abs were closely correlated with the presence of renal disease, more so than anti-dsDNA Abs. Understanding the genetic basis of this complex autoantigen-autoantibody system will provide insight into the pathogenesis of lupus in mice, which may have implications for human disease. The Journal of Immunology, 2004, 172: 5078–5085.

Mice with lupus nephritis (7–10). gp70 provides a unique system to examine autoantibody production in lupus-prone mice, because, unlike anti-nuclear Abs, the Ag availability can be quantified and potentially manipulated.

Previous linkage studies in NZ mice have shown that no single locus is responsible for serum gp70 levels. A region on chromosome 13, between 30 and 50 cM from the centromere, was linked to gp70 and gp70IC levels in NZB, NZW, and BXSB mice on a C57BL/10 or C57BL/6 background (9, 11, 12). A region on distal chromosome 4 was linked to serum gp70 levels in NZB mice on a C57BL/6 background (9), and linkage with serum gp70 and/or serum gp70IC levels has also been demonstrated on chromosome 7 in NZW and 129 mice on a C57BL/6 background (12, 13). A number of other loci have demonstrated weak linkage to gp70 and gp70IC, including chromosome 17, at the H2 complex, in non-H2-matched cohorts (8, 12, 14).

Reflection on the above data suggests that genetic background might influence the loci linked to serum gp70 production. To investigate the genetic basis of gp70 and gp70IC production in the NZ model of SLE, two experimental crosses were studied: an (NZW × BALB/c)NZW-H2 F1 × NZW backcross (W-BC) and a (NZB × BALB/c)F2 intercross (B-F2). Mice in the W-BC cohort were all H2old and mice in the B-F2 cohort all H2new.

Linkage analyses were conducted on the two crosses to determine the contribution of the NZB, NZW, and BALB/c genomes to gp70 and gp70IC production. In this study, we corroborate a number of loci already linked with gp70 production, and in addition show remarkably strong linkage to a previously unrecognized locus on chromosome 12, thus highlighting the importance of the nonautoimmune background strain on complex traits. These data are confirmed using a BALB/c mouse congenic for a 34-cM region of NZW chromosome 12 (centromere to 34 cm). We show that the anti-gp70 response is constrained by both Ag availability and strain-specific genetic factors.
Materials and Methods

Mice

(NZB × BALB/c)F2 cohort, NZB/BINJ (NZB) and BALB/cByJ (BALB/c) mice were purchased from Harlan Olac (Bicester, Oxfordshire, U.K.) and maintained in the Biological Services Unit of Imperial College Faculty of Medicine (London, U.K.). These mice were crossed, and the resulting F1 progeny were intercrossed to produce the (NZX × BALB/c)F2 cohort (F1; n = 222 female mice). Additionally, control NZB, NZW, and BALB/c female mice were obtained from the same source and studied in parallel to the B-F2 cohort.

(NZW × BALB/c.H2) × NZW cohort, NZB/BINJ, NZW/LacJ, and BALB/cByJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in the animal care facilities at the National Jewish Center for Research and Medicine and University of Colorado Health Sciences Center (Denver, CO). BALB/cByJ mice congenic for the NZW H2 locus were obtained from a cohort maintained as above (15). These mice were crossed to produce a backcross cohort, (NZW × BALB/c.H2)F1 × NZW (W-BC; n = 126 female mice).

BALB/c.NZW C12 congenic. BALB/c mice congenic for a 34-cM region of NZW chromosome 12 (centromere to 34 cM) were bred to backcross 6 BALB/c mice congenic for a 34-cM region BALB/c.NZW C12 congenic.

Genetic mapping using simple sequence length polymorphisms

Oligonucleotides flanking microsatellite repeat regions polymorphic between NZB and BALB/c (B-F2) or NZW and BALB/c (W-BC) were used to amplify genomic DNA in a standard 35-cycle PCR. The resulting PCR product was electrophoresed on polyacrylamide gels (MiniProtein II electrophoresis system; Bio-Rad, Hemel Hempstead, U.K.) at 2.25 V/mm for 90 min at 13,500 rpm at room temperature, and the serum fraction was removed. Samples were stored at −70°C. Serum gp70 levels were assayed as previously described (17). Briefly, microtiter plates were coated with goat anti-gp70, followed by incubation with serum samples. The bound gp70 was detected with an alkaline phosphatase-conjugated goat anti-gp70 Ab. The anti-gp70 Abs were assayed in a similar manner, but as the anti-gp70 Abs form ICs with the excess of serum gp70, they were precipitated with polyethylene glycol. Abs to dsDNA were assayed as described previously (18). Briefly, biotinylated plasmid DNA was added to streptavidin-coated microtiter plates and incubated with serum samples. The bound anti-dsDNA Abs were detected with an HRP-conjugated anti-mouse IgG Ab.

Assessment of glomerular disease

Kidneys from the B-F2 cohort were fixed in Bouin’s solution (three parts saturated picric acid to one part 40% formaldehyde and 5% glacial acetic acid; all VWR, Poole, U.K.) for 2–4 h, transferred to 70% ethanol, processed overnight, and embedded in paraffin wax. Two-micrometer sections were cut and stained with H&E. Kidneys were assessed using the glomerular scoring system described in Ref. 18.

Typing of Friend virus susceptibility 1 (Fv1) alleles

Polymorphisms in the Fv1 gene present between NZB, NZW, BXSB, BALB/c, and C57BL/10 mouse strains were investigated by either direct sequencing or PCR. The entire coding sequence was determined in NZB, NZW, and BXSB strains by direct sequencing using the primers GGAGCA (reverse) in NZB, NZW, BXSB, C57BL/10, and BALB/c mice.

Serum levels of gp70 and gp70IC in parental mice

In a cohort of 9-mo-old female NZB mice (n = 15), the median serum gp70 level was 41.8 μg/ml, and the median gp70IC level was 4.0 μg/ml; in age- and sex-matched NZW mice (n = 11), the median serum gp70 level was 37.5 μg/ml, and the median gp70IC level was 0.30 μg/ml. Female BALB/c mice (n = 14) had significantly lower gp70 (0.34 μg/ml; p < 0.0001) and produced minimal anti-gp70 Abs of ≤0.05 μg/ml, the lower limit of detection in the assay.

Linkage analysis and linkage map

A genome-wide scan for loci linked with serum gp70 levels in both the B-F2 and W-BC crosses revealed a highly significant area of linkage within a 7-cM region on proximal chromosome 12 (Fig. 1). Serum gp70 levels were measured at two time points (7 and 9 mo of age in the W-BC cohort; 6 and 10 mo of age in the B-F2 cohort), both of which demonstrated highly significant linkage in their respective crosses. The log of odds (LOD) scores ranged from 8.4
Linkage of serum gp70 levels to a proximal region of chromosome 12 in two independent crosses and at two time points (W-BC; 7 mo of age) to 23 (B-F2; 6 mo of age) (Table I). The degree of glomerular disease was calculated by the addition of filtration, and the data were analyzed using two statistical methods. Serum levels of gp70IC at 10 mo of age were deemed high or low based on being 25th percentile (1.0 g/ml) and 75th percentile (3.5 g/ml) of the entire data set, respectively.

Association of serum gp70IC levels with glomerular disease
As previously discussed, the presence of gp70IC is believed to have pathological significance in the development of renal disease in NZ mice. We examined this relation in the B-F2 cohort. Kidney sections were assessed for both glomerular matrix scarring and cellular infiltration, and the data were analyzed using two statistical methods. Serum levels of gp70IC at 10 mo of age were deemed high or low based on being ≥75th percentile (3.5 μg/ml) and ≤25th percentile (1.0 μg/ml) of the entire data set, respectively.

The degree of glomerular disease was calculated by the addition of

<table>
<thead>
<tr>
<th>Table I. Linkage of serum gp70 levels to a proximal region of chromosome 12 in two independent crosses and at two time points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Position of maximal linkageb</td>
</tr>
<tr>
<td>Closest marker (position)</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Strainb</td>
</tr>
<tr>
<td>pb</td>
</tr>
<tr>
<td>LOD</td>
</tr>
</tbody>
</table>

a Centimorgan positions from centromere were deduced by interval mapping, anchoring marker locations according to data from www.informatics.jax.org.

b Number of mice in which serum gp70 levels were measured.

c Strain from which gp70-modifying alleles are derived.

***, Highly significant based on permutation test.
Abs at 12 mo of age to nephritis with Fisher’s exact test, showed a weak association ($p = 0.09$).

Grouping the B-F$_2$ cohort, based on renal histological data, into high (a score of 4–7) and low (a score of 0–1) sets allowed the comparison of both the serum levels of gp70IC and anti-dsDNA IgG Abs. Both Ab species were significantly raised in the mice with a high degree of renal disease. Mirroring the results from the Fisher’s exact test, the levels of serum gp70IC were more strongly associated with renal disease ($p = 0.0003$) than anti-dsDNA IgG Abs ($p = 0.0031$) (Fig. 5).

**Investigation of Fv1 as a candidate gene for the chromosome 4 locus**

The locus on chromosome 4 had the most significant linkage, after chromosome 12, to serum gp70 levels, and had been associated with serum gp70 in a previous study of NZB × C57BL/6 crosses (9). To test the hypothesis that the locus on distal chromosome 4 encodes a structural gp70 element, we examined a cDNA sequence (X59305) derived from an NZB hepatic library that has been suggested to encode a serum gp70 (26). A nucleotide-nucleotide BLAST search also showed considerable homology of the protein motif (Interpro accession no. IPR002050). However, the BLAST search also showed considerable homology of the gp70 gene sequence to clones located on chromosomes 2 and 11. Additionally, an Ensembl WU-BLAST search on the gp70 sequence X59305 resulted in 76 chromosomal associations. In both cases, the chromosome 2 locus had the highest degree of similarity with the gp70 locus. Therefore, it is possible that the chromosome 4 locus is structural, but the alignment may be coincidental.

**Fv1** is a gene on distal chromosome 4, within the linkage region mapped in this study, that has been shown to influence retroviral replication (19). Thus, **Fv1** is a good candidate gene to explain the linkage data in NZ mice. In a study of genetic associations with gp70 and gp70IC in BXSB mice crossed with C57BL/10, no linkage to distal chromosome 4 was observed (11). Therefore, we investigated the polymorphisms in **Fv1**, comparing NZB, NZW, BXSB, C56BL/10, and BALB/c strains. The entire gene sequence of **Fv1** was determined in NZB, NZW, and BXSB mice by direct sequencing, along with the sequencing of a previously described polymorphic region (19). Additionally, the presence of a 1.3-kb deletion and downstream IAP domain (previously documented by

---

### Table II. Linkage of serum gp70 levels to regions other than chromosome 12 in B-F$_2$ and W-BC mice

<table>
<thead>
<tr>
<th>Cross/Age</th>
<th>W-BC/9 mo</th>
<th>B-F$_2$/6 mo</th>
<th>B-F$_2$/10 mo</th>
<th>W-BC/9 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>D4Mit343 (79 cM)</td>
<td>Fvl (76.5 cM)</td>
<td>Fvl (76.5 cM)</td>
<td>D9Mit9 (48 cM)</td>
</tr>
<tr>
<td>Position of maximal linkage$^d$</td>
<td>70.2–71.1 cM</td>
<td>71.8–72.9 cM</td>
<td>71.8–72.9 cM</td>
<td>54.9 cM</td>
</tr>
<tr>
<td>Closest marker (position)</td>
<td>D4Mit343</td>
<td>Fvl</td>
<td>Fvl</td>
<td>D9Mit9</td>
</tr>
<tr>
<td>Strain$^b$</td>
<td>NZW</td>
<td>NZB</td>
<td>NZB</td>
<td>NZW</td>
</tr>
<tr>
<td>p value</td>
<td>$1.4 \times 10^{-2}$</td>
<td>$3.9 \times 10^{-6}$</td>
<td>$5.0 \times 10^{-4}$</td>
<td>$1.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>LOD</td>
<td>1.3$^*$</td>
<td>5.4***</td>
<td>3.3$^*$</td>
<td>2.2$^*$</td>
</tr>
</tbody>
</table>

$^a$ Centimorgan positions from centromere were deduced by interval mapping, anchoring marker locations according to data from www.informatics.jax.org.

$^b$ Strain from which gp70-modifying alleles are derived.

$^c$ Number of mice in which serum gp70 levels were measured.

$^d$ Suggestive; *** highly significant from permutation test.

---

The two glomerular trait scores, and based on the finding that a cohort of 13 BALB/c mice had a median score of 0 (data not shown), a score of $\geq 2$ was classed as being affected. A score of 0 or 1 was classed as unaffected. Analysis of the above data using Fisher’s exact test demonstrated a significant association ($p = 0.0053$). A similar analysis, comparing serum anti-dsDNA IgG Abs at 10 mo of age to nephritis with Fisher’s exact test, showed a weak association ($p = 0.09$).

Grouping the B-F$_2$ cohort, based on renal histological data, into high (a score of 4–7) and low (a score of 0–1) sets allowed the comparison of both the serum levels of gp70IC and anti-dsDNA IgG Abs. Both Ab species were significantly raised in the mice with a high degree of renal disease. Mirroring the results from the Fisher’s exact test, the levels of serum gp70IC were more strongly associated with renal disease ($p = 0.0003$) than anti-dsDNA IgG Abs ($p = 0.0031$) (Fig. 5).

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**Fv1** is a gene on distal chromosome 4, within the linkage region mapped in this study, that has been shown to influence retroviral replication (19). Thus, **Fv1** is a good candidate gene to explain the linkage data in NZ mice. In a study of genetic associations with gp70 and gp70IC in BXSB mice crossed with C57BL/10, no linkage to distal chromosome 4 was observed (11). Therefore, we investigated the polymorphisms in **Fv1**, comparing NZB, NZW, BXSB, C56BL/10, and BALB/c strains. The entire gene sequence of **Fv1** was determined in NZB, NZW, and BXSB mice by direct sequencing, along with the sequencing of a previously described polymorphic region (19). Additionally, the presence of a 1.3-kb deletion and downstream IAP domain (previously documented by...
Serum gp70 at 6 mo of age was observed at Fv1 compared with a backcross. In this F2 cohort, the locus exhibits max-
imum linkage 7.2 cM from the centromere, which is 1.2 cM telo-
meric of D12Mit12. This proximal chromosome 12 region has not
been previously linked or associated with serum gp70 and gp70IC
levels, and therefore represents a novel locus, specific to NZ mice
and observed only in crosses with BALB/c mice. gp70ICs have
been implicated in the pathogenesis of murine lupus nephritis (7–
10). This study provides further support for this conclusion, be-
cause mice in the B-F2 cohort with serum gp70IC levels equal to
or above the 75th percentile of the entire cohort had a significantly
higher degree of renal disease than mice with gp70IC levels equal
to or below the 25th percentile (p = 0.0053). Interestingly, when
a similar analysis was conducted using serum anti-dsDNA IgG
levels, a much weaker association was observed (p = 0.086). The
correlation between renal disease and anti-gp70 Abs was further
substantiated by the high levels of gp70IC observed in mice with
more severe renal disease (Fig. 5). Additionally, the proximal lo-
cus on chromosome 12 was strongly linked with the development
of proteinuria in the W-BC and B-F2 cohorts and histological ne-
phritis in the B-F2 cohort (18).

A region on distal chromosome 4 was also linked to serum gp70
levels. The individual p values at this region were not as signifi-
cant as those on chromosome 12, but the linkage is corroborated in both
crosses. This region of linkage has been described previously
in NZB × C57BL/6 crosses (9), but not in BXSB × C57BL/10
crosses (11). Therefore, this NZ-derived region, present in both
NZB and NZW, appears to be independent of the nonautoimmune
background strain.

The contribution of the chromosome 12 region to the variance of
serum gp70 levels ranges from 32% in the W-BC to 42% in the
B-F2. The contribution of the chromosome 4 region, at the peak of
linkage, was 12%. Therefore, these two major loci contribute to

### Table III. Linkage of serum gp70IC levels in W-BC mice

<table>
<thead>
<tr>
<th>Cross/Age</th>
<th>W-BC/7 mo</th>
<th>W-BC/7 mo</th>
<th>W-BC/10 mo</th>
<th>W-BC/10 mo</th>
<th>W-BC/14 mo</th>
<th>W-BC/14 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Position of maximal linkage</td>
<td>91.2 cM</td>
<td>13.2–19.7 cM</td>
<td>14.1–14.6 cM</td>
<td>X</td>
<td>28.1–31.4 cM</td>
<td>1–2.7 cM</td>
</tr>
<tr>
<td>Closest marker (position)</td>
<td>D1Mit36 (92.3 cM)</td>
<td>D7Nde5 (23 cM)</td>
<td>D12Mit136 (13 cM)</td>
<td>X</td>
<td>D7Mit91 (28.1 cM)</td>
<td>D12Mit291 (1 cM)</td>
</tr>
<tr>
<td>n</td>
<td>123</td>
<td>123</td>
<td>123</td>
<td>123</td>
<td>123</td>
<td>123</td>
</tr>
<tr>
<td>p value</td>
<td>9.6 × 10^-3</td>
<td>7.4 × 10^-2</td>
<td>1.1 × 10^-4</td>
<td>X</td>
<td>2.4 × 10^-3</td>
<td>2.1 × 10^-5</td>
</tr>
</tbody>
</table>

### Discussion

**Genetic linkage of serum gp70 Ag levels**

We have studied the genetic basis of gp70 and gp70IC production
in NZB and NZW mice in the context of a BALB/c background.
A locus on proximal chromosome 12 in both NZB and NZW mice
was identified that is strongly linked with serum gp70 and serum
gp70IC levels. The point of maximal linkage, as determined by
interval mapping and fixing known marker positions, seemed to
differ slightly between the two cohorts. 1 cM in the W-BC cohort
vs 7.2 cM in the B-F2 cohort. This is unlikely to represent different
loci. The most accurate positional determination of the chromo-
some 12 locus is in the B-F2 cohort, a result of both the increased
numbers of mice and the increased number of recombinants com-
pared with a backcross. In this F2 cohort, the locus exhibits max-

### Table IV. Linkage of serum gp70IC levels in B-F2 mice

<table>
<thead>
<tr>
<th>Cross/Age</th>
<th>B-F2/10 mo</th>
<th>B-F2/10 mo</th>
<th>B-F2/14 mo</th>
<th>B-F2/14 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Position of maximal linkage</td>
<td>91.7 cM</td>
<td>7.2 cM</td>
<td>91.6 cM</td>
<td>10.7–11.8 cM</td>
</tr>
<tr>
<td>Closest marker (position)</td>
<td>D1Mit36 (92.3 cM)</td>
<td>D12Mit12 (6 cM)</td>
<td>D1Mit36 (92.3 cM)</td>
<td>D12Mit124 (13 cM)</td>
</tr>
<tr>
<td>n</td>
<td>154</td>
<td>154</td>
<td>178</td>
<td>178</td>
</tr>
<tr>
<td>p value</td>
<td>1.2 × 10^-3</td>
<td>1.1 × 10^-6</td>
<td>1.5 × 10^-2</td>
<td>2.8 × 10^-4</td>
</tr>
<tr>
<td>LOD</td>
<td>2.91*</td>
<td>5.94***</td>
<td>1.82</td>
<td>2.86*</td>
</tr>
</tbody>
</table>
just over 50% of the variance of serum gp70 levels. The remaining
50% of the trait variance may be influenced by both environmental
factors and other genetic effects from undetected loci, presumably
with an extremely low penetrance. Obvious candidate loci, if this
were the case, would be the numerous retroviral loci found
throughout the mouse genome. In conclusion, these linkage data
indicate that a locus on proximal chromosome 12 acts as a key
genetically controlled checkpoint for high gp70 production in
NZ mice.

The significantly increased serum gp70 levels in an BALB/
c.NZW chromosome 12 congenic confirm the role of this region in
the regulation of serum gp70 (Fig. 4). Interestingly, for a locus
with a significant effect on the variance of serum gp70, the con-
cgenic mouse had a greatly reduced median serum gp70 level com-
pared with NZW mice (1.80 vs 37.5 μg/ml). Because the environ-
mental conditions were as similar as possible between the two
cohorts, this suggests that a number of other elements from the
NZW genome are required for high levels of serum gp70.

In the current two crosses, no consistent suggestion for linkage
to gp70 and/or gp70IC was seen at the region of chromosome 13
(∼40 cM from the centromere), previously described in NZB ×
C57BL/6 (9), as Bx×6 in BXSB × C57BL/10 (11) and in NZW ×
C57BL/6.Yaa (12) crosses. This suggests that a number of other elements from the
NZW genome are required for high levels of serum gp70.

The proposed mechanism of chromosome 4 locus
The chromosome 4 locus maps in crosses of both NZB and NZW
and, unlike the locus on chromosome 12, is independent of back-
ground. We considered that a gene polymorphism might contribute
to serum gp70 either by encoding a structural gp70 gene or by a
cis-regulatory effect on structural locus. The bioinformatic data
data suggest that a sequence homology to a gp70 clone exists at the
locus on distal chromosome 4, but in conjunction with consider-
able homology to other genomic areas. Therefore, it cannot be
determined at this time if the homology to distal chromosome 4 is
purely circumstantial.

To test our second hypothesis, that the locus on chromosome 4
exerts a regulatory effect on serum gp70 levels, the surrounding
region was examined for candidate genes that may be associated
with the control of gp70. The gene Fv1 can control retroviral rep-
lication (19) and is within the region mapped. It is known that
BALB/c and C57BL/6 have a common Fv1 allele, Fv1b (19). NZB
and NZW share the Fv1nr allele (28), which encodes a protein that
is polymorphic, truncated, and functionally different compared
with the product of the Fv1b allele (28). A previous study in which
linkage to gp70 was examined in a BXSB × C57BL/10 cross
demonstrated no linkage to the distal region of chromosome 4 (11).
Based on genotype (Table V), we confirm that NZB and NZW
were identical in sequence and polymorphic features, and carry the

FIGURE 3. Correlation between serum gp70 and gp70IC levels in 10-
mo-old B-F2 mice (n = 154). The dotted line represents the serum level of
gp70, ∼1.1 μg/ml, under which minimal (<1 μg/ml) gp70IC is produced.

FIGURE 4. Serum gp70 levels in 9-mo-old BALB/c (n = 14) and
NZW.BALB/c chromosome 12 congenic mice (n = 37). Median serum
gp70 levels are shown; significance of difference was calculated with
Mann-Whitney U test.

FIGURE 5. Comparison of serum gp70IC levels at 10 mo of age (A) and anti-dsDNA IgG Abs at 12
mo of age (B) in B-F2 mice with low (0–1) and high
(4–7) glomerular histology scores. Median Ab lev-
els are shown; significance of difference was calcu-
lated with Mann-Whitney U test.
Table V. Polymorphisms in Fv1 in NZB, NZW, BXSB, C57BL/10, and BALB/c

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nucleotide/Codon Position (Based on Fv1 Sequence X97719) and/or Polymorphism</th>
<th>1.3-kb deletion</th>
<th>4902-544-bp deletion</th>
<th>IAP domain</th>
<th>Allele type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZB</td>
<td>TT/F</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Fv1&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
<tr>
<td>NZW</td>
<td>TT/F</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Fv1&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
<tr>
<td>BXSB</td>
<td>C/T/F</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Fv1&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>C/T/F</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Fv1&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
<tr>
<td>BALB/c</td>
<td>C/T/F</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Fv1&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on genotype.

Fv1<sup>mr</sup> allele. However, BXSB differed at nucleotide position 3222 from NZB and NZW, having, based on genotype, the Fv1<sup>mr</sup> allele. Therefore, BXSB and the NZ strains have different Fv1 alleles, giving support to our hypothesis that this gene is a good candidate in the genetic control of serum gp70 levels. If Fv1 is the causal gene, then the polymorphism at nucleotide position 3222 is likely to be functionally significant. No other polymorphisms in the coding sequence between NZ and BXSB were detected. The inclusion of Fv1 in the linkage analysis provides additional support for the hypothesis that Fv1 underlies the linkage on chromosome 4—an interval map placed the maximum linkage at ~4 cM proximal of Fv1, which was the most significantly linked marker in the marker map.

Proposed mechanism of chromosome 12 locus

The chromosome 12 locus associated with high serum gp70 and anti-gp70IC levels is apparent in NZ mice on a BALB/c background, but not in NZ mice on a C57BL/6 background. Therefore, it is reasonable to suggest that any gene or genes influencing these traits on proximal chromosome 12 would have to be polymorphic between BALB/c and C57BL/6. An endogenous retroviral polymerase gene, Pol7, maps within the linkage region, and is known to be polymorphic between BALB/c and C57BL/6 (29). However, a nucleotide-nucleotide BLAST (www.ncbi.nlm.nih.gov/blast) and related sequence (www.ncbi.nlm.nih.gov/entrez) search of the NZB-derived endogenous murine leukemia virus gp70 gene sequence discussed above showed no homology to the region on proximal chromosome 12. A number of transcription factor genes (E2f6, ldb2) are also situated within this region, but to date, no information on polymorphisms between BALB/c and C57BL/6 in these genes has been published. In conclusion, there seems to be no candidate genes on proximal chromosome 12 that have the equivalent structural and functional characteristics as Fv1, or indeed the endogenous retroviral loci on distal chromosome 4.

Relationship between gp70 Ag and anti-gp70ICs

The region on chromosome 12 was also associated with serum levels of anti-gp70 Abs, measured as gp70IC. However, the contribution of the region to the variance of serum gp70IC levels was considerably lower than the contribution to the variance of serum gp70 levels, with a value of ~12%. This suggests a large contribution from other loci to the gp70IC production. One area that is a strong candidate for affecting serum gp70IC levels is in the region of 92 cM on chromosome 1, at D1Mit36. The locus is linked to gp70IC levels in both the W-BC and B-F<sub>2</sub> crosses, and is in a region (Sle1, Nba2) linked with autoantibody production in murine models of SLE (21, 30). The observation that BALB/c/NZW chromosome 12 congenic mice had no significant increase in gp70IC compared with BALB/c may be a result of the absence of loci that contribute to autoantibody production outside the 34-cM region on proximal chromosome 12.

A positive correlation was observed between serum gp70 and gp70IC levels in the W-BC and B-F<sub>2</sub> cohorts. These data, combined with the shared region of linkage on chromosome 12, suggest that the production of gp70IC is driven, at least partially, by Ag availability. However, as described above, it is apparent that other genetic factors also contribute to the loss of tolerance to gp70. There seems to be a threshold level of serum gp70~1.1 μg/ml, under which minimal gp70IC is produced, a phenomenon shown previously by Haywood et al. (11) in BXSB × C57BL/10 crosses. The presence of individuals that have high serum gp70 levels and low gp70IC levels indicate there are other genetic factors that drive the production of gp70IC, and that it is not solely reliant on high levels of serum gp70 Ag.

In conclusion, we have shown that, in crosses between NZ and BALB/c mice, linkage of both gp70 and gp70IC is associated with a number of loci, including a novel locus on proximal chromosome 12. Linkage at this locus was confirmed in a BALB/c/NZW chromosome 12 congenic mouse strain. Additionally, linkage to a previously described region of distal chromosome 4 was confirmed. Further analysis of candidate genes in the regions described will give some insight into the genetic control of this autoantigen-autoantibody system, which will go some way toward an understanding of the pathogenesis of murine SLE.

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References


