The Major Locus for Mouse Adenovirus Susceptibility Maps to Genes of the Hematopoietic Cell Surface-Expressed LY6 Family

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The Major Locus for Mouse Adenovirus Susceptibility Maps to Genes of the Hematopoietic Cell Surface-Expressed LY6 Family

Katherine R. Spindler,* Amanda R. Welton,* Efrem S. Lim,* Suman Duvvuru,† Irene W. Althaus,* Jennifer E. Imperiale,* Adam I. Daoud,* and Elissa J. Chesler‡

Susceptibility to mouse adenovirus type 1 is associated with the major quantitative trait locus Msq1. Msq1 was originally mapped to a 13-Mb region of mouse chromosome (Chr) 15 in crosses between SJL/J and BALB/cJ inbred mice. We have now narrowed Msq1 to a 0.75-Mb interval from 74.68 to 75.43 Mb, defined by two anonymous markers, rs8259436 and D15Spn14, using data from 1396 backcross mice. The critical interval includes 14 Ly6 or Ly6-related genes, including Ly6a (encoding Sca-1/TAP), Ly6e (Sca-2/Tsa1), Ly6g (Gr-1), and gpihhp1 (GPI-anchored high-density lipoprotein–binding protein 1), as well as the gene encoding an aldosterone synthase (Cyp11b2). The Ly6 family members are attractive candidates for virus susceptibility genes because their products are GPI-anchored membrane proteins expressed on lymphoid and myeloid cells, with proposed functions in cell adhesion and cell signaling. To determine interstrain variation in susceptibility and produce additional resources for cloning Msq1, we assayed the susceptibility phenotype of four previously untested inbred mouse strains. Susceptibility of strain 129S6/SvEvTac was subsequently localized to the Ly6 complex region, using polymorphic genetic markers on Chr 15 in a population of 271 (129S6/SvEvTac × BALB/cJ)F1 × BALB/cJ backcross mice. We identified a major 129S6/SvEvTac susceptibility allele, Msq1129S6, on Chr 15 in the same region as Msq1129S6. The results indicate that a major host factor in mouse adenovirus type 1 susceptibility is likely to be a member of the Ly6 gene family. The Journal of Immunology, 2010, 184: 3055–3062.

The genetic background of an individual can significantly influence the outcome of viral infection. Identification of genes involved in susceptibility or resistance to viruses has increased our understanding of many viral disease processes (1). Unbiased genetic approaches have revealed specific host proteins involved in susceptibility to both RNA and DNA viruses, such as West Nile virus and mouse CMV (2–5).

Inbred and outbred strains of mice vary significantly in their susceptibility to mouse adenovirus type 1 (MAV-1) (6–8). MAV-1 has similarities to human adenoviruses in structure, genome organization, and some aspects of pathogenesis (9). Both MAV-1 and human adenoviruses cause acute and persistent infections with high morbidity and mortality in immunocompromised hosts. Respiratory infection by both mouse and human adenoviruses results in chemokine upregulation (reviewed in Ref. 10). MAV-1 primarily infects endothelial cells, which is only rarely seen for human adenoviruses. The endotheliotropism is particularly marked in the brain, spinal cord, and spleen (7, 11, 12), and MAV-1 causes hemorrhagic encephalomyelitis accompanied by breakdown of the blood–brain barrier and altered expression of tight junction proteins (6, 7, 13). In addition, MAV-1 targets monocytes and macrophages, which are also effectors of the host response to MAV-1 infection (14). We are investigating the mechanisms involved in MAV-1 pathogenesis, using a variety of methods, and we have shown that both adaptive and innate immune responses play a role in the viral disease process (10, 14–16). We are using a positional cloning approach to identify the important host genes involved in susceptibility to mice of MAV-1.

Susceptibility to MAV-1 is a dominant quantitative trait, with a quantitative trait locus (QTL), Msq1, mapping to chromosome (Chr) 15 (logarithm of odds [LOD] score = 21) (17). Msq1SJL accounts for 40% of the trait variance between the susceptible SJL/J strain and resistant BALB/cJ strain. Initially, the 2 LOD confidence interval for Msq1SJL was 18 Mb and included ~300 predicted genes, including Hsf1 (the gene encoding heat shock factor 1 protein), Sla1 (Src-like adapter protein 1), and the Ly6 gene-family complex (lymphocyte Ag 6). The Ly6 genes are particularly attractive as candidate genes for susceptibility because they encode GPI-anchored cell-surface proteins (18). There are >20 predicted Ly6 or Ly6-related genes in this interval of Chr 15, and protein functions or expression patterns are known for some. Ly6 proteins are variably expressed on different cell types, including lymphoid and nonlymphoid cells, and during hematopoietic cell differentiation. Some Ly6 proteins are involved in lymphocyte adhesion, T cell responses, and the IFN response. The
chicken Ly6E gene has been identified as a candidate gene for resistance to the avian herpesvirus Marek’s disease virus (19). Recently, two different approaches have identified Ly6 or Ly6-related gene involvement in HIV-1 susceptibility. A whole-genome linkage analysis using cellular susceptibility to HIV in vitro identified the Ly6 locus on human Chr 8q24 (20). A small interfering RNA screen identified 250 HIV-dependency factors, 3 of which were members of the Ly6 family: Gm1, Ly6D, and Ly6D4 (21). In a similar approach, Ly6E was identified as 1 of 305 human proteins that affect West Nile virus infection (22).

To determine which of the candidate gene(s) in the Mrq1 locus correlates with susceptibility to MAV-1, we used fine mapping and haplotype analysis to reduce the interval from 18 Mb to 0.75 Mb, a region that encompasses 14 Ly6 or Ly6-related genes. The Ly6 gene complex in mice has two major haplotypes, Ly6a and Ly6b, that code for two lymphocyte specificities with distinct inbred mouse strain distribution patterns (23–25). These strain distribution patterns correlate with known susceptibility or resistance to MAV-1 among inbred strains (8). We therefore identified additional strains of mice that are susceptible or resistant to MAV-1 to extend this correlation and develop additional mapping tools. We analyzed progeny of a backcross between BALB/cJ mice and one additional MAV-1–susceptible mouse strain, 129S6/SvEvTac. We identified a QTL allele, Mrq1, that maps to the same region of Chr 15 as Mrq1 (LOD = 46). Taken together, the results provide strong evidence that susceptibility to MAV-1 is encoded by one or more members of the Ly6 gene complex.

Materials and Methods

Viruses and mice

Wild-type MAV-1 (26) was propagated and titrated as described (27). All virus stocks used for phenotyping were aliquots from a single preparation that were thawed only one time, for preparation of inoculum. All mice were obtained from The Jackson Laboratory (Bar Harbor, ME) except for 129S6/SvEvTac (129S6), which were obtained from Taconic Farms (Germantown, NY). All animal work complied with relevant federal and institutional policies. Mice were maintained in microisolator housing with food and water provided ad libitum. A selective phenotyping scheme was used on 1140 backcross progeny of BALB/cJ and SJL/J or 129S6 mice by 271 backcross mice genotyped at five markers. General linear modeling (ANOVA) of the allelic effect was performed using the R/qtl software. SSLP markers used in the QTL analysis were D15Mit13, D15Mit66, D15Snp101, D15Mit70, and D15Snp107. Individual markers were quality tested for difference from the expected 1:1 ratio, using the x2 test for Mendelian segregation. The D15Mit66 marker showed significant deviation from the 1:1 ratio (p = 7.87 × 10−13) with a surplus of BALB/cJ homozygote allele calls relative to heterozygotes. None of the other markers had significant deviation from expectation (p > 0.05). Mouse brain virus loads were entered as the background-subtracted average ELISA value of triplicate measurements from each mouse. QTL mapping was performed using the R/qtl scanone function with single-marker regression and the imputation method, using 271 backcross mice genotyped at five markers. General linear modeling (ANOVA) of the allelic effect was performed using the R/qtl scanone function at the marker nearest to the QTL peak.

Results

Fine mapping of the Mrq1 interval

The 2-LOD confidence interval previously identified for Mrq1 on Chr 15 was estimated to be from 39 to 54 cM, corresponding to ~65–78 Mb on the physical map (17). We determined the MAV-1 susceptibility phenotypes for 256 (BALB/cJ × SJL/J)F1 × BALB/cJ backcross progeny and then genotyped for markers on all 20 mouse chromosomes. Forty backcross mice were recombinant across the Chr 15 2-LOD confidence interval defined by two markers used in the original analysis, D15Mit270 (63.3 Mb) and D15Mit70 (81.0 Mb). An additional microsatellite marker that had been previously described (D15Mit211, 66.6 Mb) was supplemented with two others that we designed, D15Snp101 and D15Snp54 (Table I), to further genotype the recombinant mice. Five of the 256 backcross mice were recombinant across the interval. To fine map the genetic location of Mrq1, we expanded the backcross, collecting 1140 additional mice. A selective phenotyping scheme was used: genotypes were determined for all progeny mice at D15Snp101 and D15Snp54; subsequently, only mice recombinant across this interval were infected with MAV-1 for susceptibility phenotype measurement. We identified 19 additional backcross mice that were recombinant across the interval (Fig. 1); 16 of these had phenotypes concordant with their genotypes. The three mice with discordant genotypes and phenotypes are discussed below. Taken together, the original and the expanded backcrosses yielded 21/1396 recombinants (1.5%) across the Mrq1 interval from 72.56 to 75.85 Mb of Chr 15.

To further refine the candidate interval, we developed additional microsatellite markers and identified SNP markers that are polymorphic for BALB/cJ and SJL/J. The 21 recombinant mice were each genotyped using 2 or more of these 9 markers (Fig. 1); from these results we deduced a maximal 0.75-Mb interval for the Mrq1 QTL. One individual had a crossover between rs8259436 and 3056 MAJOR LOCUS FOR MA V-1 SUSCEPTIBILITY MAPS TO LY6 FAMILY

nomic interval of interest, using the Tandem Repeats Database (http://tandem.iub.edu/cgi-bin/drd/trdrb.exe) (29). Primers were designed to amplify regions of ~200 nt from template DNAs for the three mouse strains. Primer pairs that amplified newly identified polymorphic PCR products used in this work are listed in Table I.

Single nucleotide polymorphisms (SNPs) potentially polymorphic for the mouse strains and interval of interest were initially identified using SNP databases extant in 2005–2006, GNF3 and Celera2 (30, 31). The SNPs are now compiled in the Mouse Phenome Database (www.jax.org/phenome/SNP) and are shown in Fig. 1 by numbers with an "rs" prefix. Further SNP haplotype analysis of additional resistant and susceptible strains was done using the Center for Genome Dynamics Mouse SNP Database v. 1.3 (http://c gd.jax.org/cgdsnpdb/).

DNAs were isolated from tail snips and amplified by PCR in 20-μl reactions as described (32). Samples were electrophoresed on 7% polyacrylamide gels. Alternatively, DNA samples were analyzed by the University of Michigan Sequencing Core (Ann Arbor, MI), using fluorescently labeled primers. For SNP analysis, only an aliquot of the PCR reaction was electrophoresed on a gel. The remainder of each sample was purified using QIAquick PCR Purification Kit columns (Qiagen, Valencia, CA), and the DNA was then sequenced by the University of Michigan Sequencing Core.

Statistical analysis and QTL mapping

QTL analysis was performed using R/qtl software. SSLP markers used in the QTL analysis were D15Mit13, D15Mit66, D15Snp101, D15Mit70, and D15Snp107. Individual markers were quality tested for difference from the expected 1:1 ratio, using the x2 test for Mendelian segregation. The D15Mit66 marker showed significant deviation from the 1:1 ratio (p = 7.87 × 10−13) with a surplus of BALB/cJ homozygote allele calls relative to heterozygotes. None of the other markers had significant deviation from expectation (p > 0.05). Mouse brain virus loads were entered as the background-subtracted average ELISA value of triplicate measurements from each mouse. QTL mapping was performed using the R/qtl scanone function with single-marker regression and the imputation method, using 271 backcross mice genotyped at five markers. General linear modeling (ANOVA) of the allelic effect was performed using the R/qtl scanone function at the marker nearest to the QTL peak.
Table I. New microsatellite loci used in this study

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<tr>
<th>Locus</th>
<th>Map Position</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Polymorphic Between</th>
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<tbody>
<tr>
<td>D15Spn101</td>
<td>72,556,431</td>
<td>5’-TCAGGCAAGAAGCTGCAAGG-3’</td>
<td>5’-ACAGTTGTCCCCCGTTCTGCC-3’</td>
<td>BALB/cJ and SJL/J</td>
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<tr>
<td>D15Spn106</td>
<td>72,849,859</td>
<td>5’-ATCCCCACCCGAGTGAAGAAGAC-3’</td>
<td>5’-TTTCATGACCCGTTGAGCAG-3’</td>
<td>BALB/cJ and 129Sv</td>
</tr>
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<td>D15Spn14</td>
<td>75,432,404</td>
<td>5’-GGCTGGGAGAGAGACTTCA-3’</td>
<td>5’-CAAGTTGAAAGGGGATCAC-3’</td>
<td>BALB/cJ and 129S6</td>
</tr>
<tr>
<td>D15Spn54</td>
<td>75,850,197</td>
<td>5’-CCTGCTGGGAGAGAGACTTCA-3’</td>
<td>5’-CAAGTTGAAAGGGGATCAC-3’</td>
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</table>

*Map position indicated is the first nt at the centromeric side of the repeat (based on NCBI Build 37, July 2007).

FIGURE 1. Genetic mapping of $Mqj$. The top line shows SSLP and SNP markers used in fine mapping (BALB/cJ × SJL/J)F1 × BALB/cJ backcross progeny in the Chr 15 interval between 72.56 and 75.85 Mb; physical positions of each marker are indicated in parentheses. The X symbols indicate the number of mice with genotypic recombination within each subinterval. The lower line is an expansion of the deduced critical interval with predicted progeny in the Chr 15 interval between 72.56 and 75.85 Mb; physical positions of each marker are indicated in parentheses. The X symbols indicate the susceptibility of additional inbred mouse strains to MAV-1

The Ly6 complex has long been known to have two haplotypes, $Ly6^a$ and $Ly6^b$, that code for two lymphocyte specificities, Ly6.1 and Ly6.2, respectively, determined by allelism to cell-membrane Ags (23–25). The distribution pattern of these Ly6.1 and Ly6.2 specificities among inbred mouse strains correlates with susceptibility that we had previously identified for a number of Ly6.2 specificities among inbred mouse strains. For example, Ly6.1 strains include BALB/c, A/J, A. SW, and C3H/HeJ (23), which are resistant to MAV-1 (8, 17). Ly6.2 specificities among inbred mouse strains correlates with susceptibility that we had previously identified for a number of inbred strains. For example, Ly6.1 strains include BALB/c, A/J, A. SW, and C3H/HeJ (23), which are resistant to MAV-1 (8, 17). Ly6.2 strains include SJL/J, SWR/J, FVB/NJ, and PL/J had haplotypes in the region identical to those found in the resistant strains BALB/cJ, A/J, and C3H/HeJ (data not shown). This observation is consistent with our inability to find known SNPs or design microsatellite markers that detect polymorphisms between BALB/cJ and SJL/J mice for the interval from 72.85 to 74.68 Mb. In contrast, in the region from 74.6 to 75.3 Mb, the four susceptible strains had identical haplotypes to each other that were different from the shared haplotypes of the three resistant strains (data not shown). With a denser SNP database (Center for Genome Dynamics Mouse SNP Database v. 1.3), the haplotype identity persisted from 73.4 to 74.5 Mb (data not shown). The haplotype analysis did not reduce the number of candidate genes in the interval.

Susceptibility of 129S6 mice maps to Chr 15

To determine whether susceptibility of 129S6 mice maps to the same region of Chr 15 as susceptibility of SJL/J mice, we crossed 129S6 and BALB/cJ mice. Progeny $F_1$ animals were infected with MAV-1 and had susceptibility phenotypes (brain virus loads) intermediate between the parental phenotypes (data not shown). This result is similar to the case for (BALB/cJ × SJL/J)F1 mice and rs3662946, defining the left end of the interval at 74.68 Mb, and four individuals had crossovers between rs3717260 and D15Spn14, defining the right end of the interval at 75.43 Mb.

Fifteen candidate genes in the 0.75-Mb interval were identified using Ensembl and are depicted in Fig. 1 and listed in Table II (33–43). Eight are genes encoding Ly6 family proteins ($Ly6e$, $Ly6i$, $Ly6a$, $Ly6c1$, $Ly6c2$, $Ly6g$, $Ly6f$, and $Ly6h$). Six genes ($gpihbp1$ and five single transcripts of unknown association, 2010109103Rik-201, 1830127L07Rik, BC025446, AC116498.15, and 9030619P08Rik) encode predicted proteins that have LY6/uPAR sequences in a large protein family related. LY6/uPAR sequences are found in a large protein family related. $Ly6a$-related genes are indicated by black triangles, $Ly6b$-related genes by gray triangles, and the other gene by a white triangle. Genbank transcript designations for the Ly6-related genes ($Ly6R1$–5) are given in Table II. Genes transcribed from the top (+) strand point to the right, and genes transcribed from the bottom (−) strand point to the left. Numbers below the line indicate the physical map position in megabases.
mice from two types of F1 parents were plotted separately (data not shown). The distribution was similar when phenotypes of backcross progeny, and determined their susceptibility phenotypes. Fig. 3 shows the distribution of phenotypes of the entire backcross population, and using single-marker regression and the imputation method (256 markers on Chr 15 that differentiate between 129S6 and BALB/cJ mice. We performed QTL analysis on the 271 backcross mice, determined by a single major locus.

We obtained 271 backcross mice, consisting of both (129S6 × BALB/cJ)F1 × BALB/cJ and (BALB/cJ × 129S6)F1 × BALB/cJ progeny, and determined their susceptibility phenotypes. Fig. 3 shows the distribution of phenotypes of the entire backcross population. The distribution was similar when phenotypes of backcross mice from two types of F1 parents were plotted separately (data not shown). The phenotype distribution for the backcross mice is consistent with susceptibility of 129S6 mice being a quantitative trait determined by a single major locus.

We determined genotypes of the 271 backcross mice for five markers on Chr 15 that differentiate between 129S6 and BALB/cJ mice. We performed QTL analysis on the 271 backcross mice, using a QTL mapping package for the statistical software R, R/qtl, as previously described (17). We observed an association of susceptibility with markers on Chr 15. A one-way scan for QTLs using single-marker regression and the imputation method (256 imputations using 5000 permutations and a 2-cM step interval) of the mapping analysis showed that viral load is associated with a major effect QTL on Chr 15. The peak marker was D15Spn101 from single-marker analysis, and imputation mapping showed the effect maxima on Chr 15 at ~69 Mb with a peak LOD of 46.2. The results from fitting the candidate QTL using ANOVA showed the percentage of phenotypic variation explained by the QTL at 69 Mb on Chr 15 analyzed alone was 54% (F11, 269) = 331; p = 9.01 × 10−49, LOD = 47.3. This interval on Chr 15 for Msq1129S6 encompasses the interval we have previously identified for Msq11SJL.

**Discussion**

To understand the contribution of genetic variation in host defense pathways to adenoviral pathogenesis and to identify which host genes play a role in MAV-1 susceptibility, we have used a positional cloning approach. An 18-Mb region of mouse Chr 15 was initially identified as the region with the major QTL for MAV-1 susceptibility; this region encoded ~300 predicted genes (17). We used backcross mice, additional polymorphic markers, and SNP haplotype identity to reduce the size of the critical interval to 0.75 Mb. This region encodes 14 Ly6 or Ly6-related genes and an aldosterone synthase gene. We cannot eliminate aldosterone synthase as a candidate gene, but we believe that Ly6 family members are more likely for reasons based on expression and function, discussed below. The fine mapping eliminated the candidate genes Hsf1 and Slal, which were previously considered candidates in the 18-Mb interval based on their biological function, as well as several Chr 15 Ly6 and Ly6-related genes, such as Ly6d, Ly6k, Lynx1, Slurp1, and Psca.

In the (BALB/cJ × SJL/J)F1 × BALB/cJ backcross analysis, 3 of 19 mice that were recombinant across the interval delineated by D15Spn101 and D15Spn54 had phenotypes for susceptibility to MAV-1 that were discordant with their genotypes for markers within the interval. The mice were resistant to MAV-1, as measured by brain viral load, and yet their genotype was heterozygous (BALB/cJ/SJL/J) at the four markers in the interval from 74.80 to 75.28 Mb. Because susceptibility to MAV-1 is dominant (17), mice with the heterozygous genotype should be susceptible. In each case, the phenotype-genotype discordance in these three individuals is in the direction of unexpected resistance. The virus did not replicate to high levels in their brains, despite being infected with virus. We have previously seen such false-negative

**FIGURE 2.** MAV-1 susceptibility of inbred strains of mice. Brain viral loads were determined by capture ELISA. Each brain homogenate was assayed in triplicate, and each bar represents the average of the background-subtracted triplicate measurements per homogenate with SD. Samples were standardized to a virus stock positive control included in each assay. OD450, OD at 450 nm. Strain abbreviations: SJL, SJL/J; SWR, SWR/J; 129S6, 129S6/SvEvTac; FVB, FVB/NJ; NOD, NOD/ShiLtJ; B6, C57BL/6J; CH, C3H/HeJ; 129P3, 129P3/J; BALB, BALB/cJ.

<table>
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<td>Ly6-related</td>
<td>Lty6Rb</td>
<td>74.71, +</td>
<td>201010993Rik, interectin (41)</td>
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<tr>
<td>Ly6-R2</td>
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<td>AC116498.15-201</td>
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<td>Ly6-R5</td>
<td>75.26, +</td>
<td>9030619P0Rik</td>
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<tr>
<td>Gpibp1</td>
<td>75.43, +</td>
<td>GPI-anchored HDL-binding protein 1, expressed in heart, lung, liver (42)</td>
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<td>Other</td>
<td>Cyp11b2</td>
<td>74.69, +</td>
<td>Cytochrome P450 11b2, aldosterone synthase</td>
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*Natralt Build 37. Position listed is the start of transcription.

Ly6-related 1–5; designation given in this paper for purpose of discussion.

Mouse Genome Database (www.informatics.jax.org, August 2009) (43).

Gr-1, granulocyte differentiation Ag; HDL, high-density lipoprotein; Sca, stem cell Ag; TAP, T cell-activating protein; TSA-1, thymic shared Ag-1.
occurrences of low viral load in known genetically susceptible mice, at a frequency of 2–4% (K.R. Spindler, unpublished data).

An example of a single animal of this type can be seen for the susceptible SJL/J mice assayed in Fig. 2. These events may be due to experimental failures in the infection procedure. A second possibility is that one or more of these three mice do not represent a false-negative phenotyping error but were truly MAV-1 resistant, despite being genetically heterozygous for the interval. That is, cumulative resistant alleles at genes elsewhere in the genome may be sufficient to produce the resistant phenotype. Susceptibility is a quantitative trait, with multiple candidate loci identified in the initial QTL analysis (17). For example, a locus on Chr 5 has an additive effect on the phenotype. We favor false-negative infection as the most likely explanation because we did not obtain any mice with discordance as unexpected susceptibility. However, the number of discordant individuals was small, and further analysis would be needed to resolve the possible explanations.

We analyzed additional strains for their MAV-1 susceptibility phenotype by measuring brain virus loads by capture ELISA. For strains that had been previously assayed for susceptibility by LD50 rather than ELISA, the ELISA and LD50 results were in agreement (SWR/J, C3H/HeJ, and 129P3/J) (8). We also identified additional susceptible and resistant strains, using ELISA. We found that the strain distribution pattern of Ly6.1 and Ly6.2 specificities among inbred strains correlated with susceptibility to MAV-1. That is, Ly6.1 strains include BALB/c, A/J, A.SW, and C3H/HeJ (23); all of these are resistant to MAV-1 (8). Ly6.2 strains include SJL, SWR, PL/J, and 129Sv (23, 25), and these are susceptible to MAV-1.

Analysis of a backcross of a second susceptible strain (129S6) with the resistant BALB/cJ strain showed that susceptibility to MAV-1 mapped to the same interval on Chr 15 as did SJL/J susceptibility. This Msq1129S6 QTL alone accounts for 54% of the variation in susceptibility phenotype. The result that susceptibility in an additional strain maps to the same locus as Msq1129S6 enabled us to use SNP haplotype analysis in conjunction with recombinant mouse analysis to confirm the critical interval. The information also enabled us to produce bacterial artificial chromosome-based transgenic mice from a 129S6 library (T.-H. Hsu and K.R. Spindler, unpublished data). Analysis of these mice for dominant MAV-1 susceptibility may reduce the candidate interval and confirm unpublished observations). Analysis of these mice for dominant MAV-1 susceptibility may reduce the candidate interval and confirm unexplained susceptibilities. This Msq1129S6 locus established by the International Committee on Standardized Genetic Nomenclature for Mice, as found in the Mouse Genome Database (www.informatics.jax.org, August 2004 and did not make extensive use of the emerging annotated mouse genome sequence (18). Furthermore, even in the current literature, references are made to populations of cells based on old designations that are ambiguous, such as Ly6A/E and Ly6G/C. The Gr-1 antigenic specificity of mAb RB6-8C5 was first reported to be shared by the Ly6c and Ly6g gene products, with Ly6C and Ly6G being expressed on distinct cell types (52).

However, a more recent study indicates that for this Ly6G Ab there is no cross-reactivity with Ly6C on multiple hematopoietic cell types (53). To assist in clarifying the Ly6 gene family nomenclature, in this discussion we use the nomenclature of genes in the Ly6 locus established by the International Committee on Standardized Genetic Nomenclature for Mice, as found in the Mouse Genome Database (www.informatics.jax.org, August 2009) (43). In addition, five Ly6-related transcripts have been identified solely by genomic sequence annotation within the critical interval for MAV-1 susceptibility (Table II). These Ly6-related transcripts are discussed below, using abbreviated names, Ly6R1–Ly6R5, because they have predicted proteins that all have Ly6/uPAR domains (data not shown).

The mRNA sequences of Ly6R1–5 are distinct from one another and from the characterized Ly6 genes. Predicted proteins of the Ly6R1, Ly6R2, and Ly6R3 transcripts are noted in the Mouse Genome Database (www.informatics.jax.org; August 2009) (43) as having Ly6/uPAR domains, that is, sequences with 10 cysteine residues in a consensus pattern with the C-terminal cysteine followed by an asparagine (44, 45). Ly6R2 lacks a predicted initiator methionine, but we have preliminary evidence of a complete Ly6R2 cDNA with an initiator methionine from SJL/J mice (M.T. Stier and K.R. Spindler, unpublished observations). We analyzed the predicted protein sequences of the other two transcripts, Ly6R4 and Ly6R5, and noted that the entire Ly6/uPAR motif is found in the open reading frame for Ly6R4. The protein predicted from Ly6R5 terminates soon after the fifth cysteine residue; however, downstream in an alternate reading frame we found a sequence corresponding to the remainder of the
LY6 homology (data not shown). Thus, it is possible that Ly6R5 is in the process of becoming a pseudogene. Few expression or functional data are available for the five Ly6-related transcripts in the Chr 15 interval. Ly6R1 encodes a protein that has been identified as lectin, an intestine-specific protein involved in apoptosis of intestinal epithelial cells (41).

Ly6 and Ly6-related genes are found in other vertebrates, including chickens, rats, and humans. In humans, 11 Ly6-related genes are found on Chr 8q24, syntenic to Chr 15 (54); in both mouse and humans, Ly6-related genes are also found on other chromosomes. The mouse Chr 15 interval that we have identified for susceptibility to MAV-1 has more genes and is longer than the corresponding human interval on Chr 8q24. That is, in the 0.61-Mb between mouse Ly6e and Ly6h there are 10 Ly6 and Ly6-related genes (Fig. 1), but there are no predicted genes with sequence similarity to Ly6 genes in the corresponding 0.14 Mb of human Chr 8 between Ly6e and Ly6h (55). Notably, Ly6A and Ly6C proteins do not have human orthologs.

Ly6 proteins encoded in the critical interval for MAV-1 susceptibility are expressed on cells that either are targets of MAV-1 (monocytes/macrophages and endothelial cells) (14, 56) or are lymphoid or myeloid cells (or their precursors) that are effectors in innate and adaptive immunity (Table II), making them strong candidates for involvement in susceptibility. Differences in sequence or expression of these proteins between susceptible and resistant strains may underlie the susceptibility differences among mouse strains. Ly6l, Ly6C1, and Ly6C2 are expressed on monocytes, macrophages, and dendritic cells (reviewed in Ref. 18); strain differences in expression patterns on Ly6.1 and Ly6.2 strains have been reported for Ly6C (although it is not known whether these differences were for Ly6C1 or Ly6C2 expression) (51). As measured by immunohistochemistry, the Ly6A protein is prominently expressed in brain vascular elements in Ly6.2 strains (C57BL/6 and AKR/J) (57, 58). These authors did not definitively show that the Ab staining was specific to endothelial cells, but it is consistent with endothelial expression of Ly6A. In contrast, no expression is seen in vascular elements of Ly6.1 strains (BALB/c and C3H/HeJ) using the same Ab (58). It will be informative to determine whether similar Ly6A expression differences are seen for additional Ly6.1 (MAV-1–resistant) and Ly6.2 (MAV-1–susceptible) strains, as this may be a cause or effect of differences in MAV-1 susceptibility.

Alterations in effector functionality of lymphoid or myeloid cells might be caused by Ly6 gene differences and lead to susceptibility to MAV-1. Ly6E is expressed on intrathymic cells that give rise to all the thymic subsets; it is differentially expressed during T cell development and is found on immature T cells and peripheral B cells (59, 60). Ly6A is expressed on embryonic and fetal aorta and liver, and on adult hematopoietic stem cells (reviewed in Ref. 55). Ly6A is differentially expressed on hematopoietic stem cells in Ly6.1 and Ly6.2 strains, with ~100% of cells and 25% of cells being positive, respectively. This is reminiscent of the different levels of expression of Ly6A in brain vascular elements, described above. Both T and B lymphocytes are important for control of MAV-1 infection (15, 16). It is possible that functional differences in T cell- and/or B cell-mediated host response to MAV-1 infection result from differences in Ly6E, Ly6A, or less well characterized Ly6 proteins in susceptible and resistant strains. Little is known about the physiological function of the Ly6 proteins, but they are thought to be involved in cell–cell adhesion and signaling (reviewed in Refs. 18, 55). Functional roles for LY6 proteins in disease pathogenesis that may relate to viral disease susceptibility are suggested by several findings. Expression of LY6 proteins is induced on many mouse tissues in the animal, upon antigenic stimulation (reviewed in Ref. 18). During infection of mice by lymphocytic choriomeningitis virus, Ly6A expression increases on CD4+ and CD8+ T cells and persists at a high level on memory T cells (61). Inflammatory monocytes express high levels of Ly6C and are recruited to sites of inflamed tissue during infection by viruses, bacteria, and parasites, in contrast to resident monocytes, which are Ly6Cd6e and rarely migrate to inflamed sites (36, 62–65). Both in vivo and in vitro stimulation of immune cells to IFNs increases expression of Ly6 genes. For example, both type I and type II IFNs stimulate Ly6 expression on T and B lymphocytes (66–68). IFNs also stimulate nonimmune cells: IFN-γ stimulates Ly6E expression in kidney (69) and on inflamed intestinal epithelial cells in murine models of colitis (70). Because IFN production is a major innate immune response to viral infection, it is reasonable to hypothesize that viral infection may result in altered expression of LY6 proteins. We suggest that this may underlie susceptibility to viral infections caused by Ly6 or Ly6-related genes.

In addition to our findings that Ly6 genes underlie the QTL for susceptibility to MAV-1, studies have implicated Ly6 genes for susceptibility to three other viruses. The chicken Ly6E locus is a major candidate for susceptibility to Marek’s disease virus, a herpesvirus, based on several experimental approaches (19, 71). The human Ly6 locus was identified in a whole-genome analysis of lymphoblastoid cell susceptibility to HIV-1 (20), and three Ly6 or Ly6-related genes were identified in a small interfering RNA screen for host factors required by HIV-1 (21). Ly6E was identified as a host susceptibility factor for West Nile virus in a similar small interfering RNA screen (22). Nothing has been reported about the mechanism by which Ly6 genes contribute to infection by these or other viruses. In fact, infection of Ly6a knockout mice by lymphocytic choriomeningitis virus demonstrated that Ly6A is dispensable for normal T cell function in acute and memory responses (61). We found no difference in susceptibility to MAV-1 infection between Ly6a knockout mice and BALB/c controls at either high or low virus doses (105 or 102 PFU, respectively) (I.W. Althaus and K.R. Spindler, unpublished observations). Given the number of Ly6-related proteins and the variety of cell and organ types in which these genes are expressed, the mechanisms may be quite different for various viruses. However, the findings that susceptibility to viruses from three different families maps to Ly6 genes strongly suggest that the Ly6 gene products are key players in virus–host interactions.

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Disclosures

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