The Journal of Immunology

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J Immunol 2006; 176:5478-5485; ;
doi: 10.4049/jimmunol.176.9.5478
http://www.jimmunol.org/content/176/9/5478

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cmv4, a New Locus Linked to the NK Cell Gene Complex, Controls Innate Resistance to Cytomegalovirus in Wild-Derived Mice[1]

Sonia Girard Adam,*† Anouk Caraux,‡ Nassima Fodil-Cornu,*† J. Concepcion Loredo-Osti,*† Sarah Lesjean-Pottier,‡ Jean Jaubert,§ Ivan Bubic,¶ Stipan Jonjic,¶ Jean-Louis Guénet,§ Silvia M. Vidal,[2,3]*† and Francesco Colucci[2,4]‡

CMV can cause life-threatening disease in immunodeficient hosts. Experimental infection in mice has revealed that the genetically determined natural resistance to murine CMV (MCMV) may be mediated either by direct recognition between the NK receptor Ly49H and the pathogen-encoded glycoprotein m157 or by epistatic interaction between Ly49P and the host MHC H-2D^k. Using stocks of wild-derived inbred mice as a source of genetic diversity, we found that PWK/Pas (PWK) mice were naturally resistant to MCMV. Depletion of NK cells subverted the resistance. Analysis of backcrosses to susceptible BALB/c mice revealed that the phenotype was controlled by a major dominant locus effect linked to the NK gene complex. Haplotype analysis of 41 polymorphic markers in the *Ly49h* region suggested that PWK mice may share a common ancestral origin with C57BL/6 mice; in the latter, MCMV resistance is dependent on Ly49H-m157 interactions. Nevertheless, PWK mice retained viral resistance against m157-defective mutant MCMV. These results demonstrate the presence of yet another NK cell-dependent viral resistance mechanism, named *Cmv4*, which most likely encodes for a new NK activating receptor. Identification of *Cmv4* will expand our understanding of the specificity of the innate recognition of infection by NK cells. *The Journal of Immunology,* 2006, 176: 5478–5485.

Infection with human CMV is a common cause of congenital disorders and can be life-threatening in organ transplant as well as AIDS patients (1–4). CMVs are strictly species specific; however, infection of mice with murine CMV (MCMV)[5] provides an excellent experimental model with which to study the genetics and pathophysiology of the host immune response (5–7). Scoring the innate immune response in terms of viral titers in the organs of infected mice 2–5 days after infection gives an unambiguous outcome that depends on the genetic makeup of the host (8, 9). Whereas most mouse strains allow uncontrolled viral growth in multiple organs, some mouse strains such as C57BL/6 (B6) and MA/My can effectively control early viral replication (8–10). A complex network of cells, soluble factors, cellular receptors, and intracellular signaling pathways organize the innate response against the virus (7, 11). NK cells, however, play a central and nonredundant role in this process (12–14). Studies of natural variation in host resistance or susceptibility to MCMV infection have clarified several aspects of the function of NK cells in the innate immune response.

In B6 and MA/My mouse strains innate resistance to MCMV is controlled by alternative loci, named *Cmv1* and *Cmv3*, which reside on the NK cell gene complex (NKC) on distal mouse chromosome 6 (9, 10, 15). The NKC encodes many NK cell receptors, including activating and inhibitory members of the Ly49 family of MHC class I receptors (16–18). In B6, *Cmv1* encodes the activating NK cell receptor Ly49H (19–21). Ly49H binds to m157, a viral MHC class I-like protein expressed at the surface of infected cells during the early phase of infection (22, 23). In MA/My, *Cmv3*-mediated innate resistance is expressed in epistatic interaction with *H-2^k* (15). In this model, the activating receptor Ly49P was shown to interact with H-2-D^k only on the MCMV-infected cells (15). Thus, activating Ly49 receptors seem to recognize infection and trigger NK cells to kill infected cells, providing a mechanism of natural host resistance.

The use of classical laboratory mouse stocks sets serious limitations, as B6 and MA/My strains represent apparent exceptions. All of other strains tested either do not possess *Ly49h* or do not coexpress *Ly49p* and *H-2^k* and are consequently susceptible to MCMV (15, 24). Moreover, other Ly49 receptors exist that can bind to m157, but the outcome of this interaction is thought to rather facilitate viral replication. One such receptor is the Ly49I inhibitory receptor on NK cells of the susceptible 129/J strain, where m157 may in fact switch off NK cells upon viral recognition (22). In contrast, most MCMV strains isolated from wild mice

*Department of Human Genetics and †McGill Center for Host Resistance, McGill University, Montreal, Canada; ‡Unit of Cytokines and Lymphoid Development, Department of Immunology, Pasteur Institute, Paris, France; §Unit of Mammalian Genetics, Department of Developmental Biology, Pasteur Institute, Paris, France; and ¶School of Medicine, University of Rijeka, Rijeka, Croatia

Received for publication December 12, 2005. Accepted for publication February 15, 2006.

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[1] This work was supported by grants from Institute Pasteur (to F.C., A.C., S.L.-P., J.J., and J.-L.G.), Ligue National Contre le Cancer and Institut National de la Santé et de la Recherche Médicale (to F.C., A.C., and S.L.-P.), Canadian Institutes for Health Research (to S.M.V., S.G..A., N.F.-C., and J.C.L.-O.), and Fonds France-Canada pour la Recherche (to S.M.V. and F.C.). The work of S.J. and I.B. was supported by the Croatian Ministry of Science and Education.

[2] These two authors contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Silvia M. Vidal, Department of Human Genetics, Department of Microbiology and Immunology, McGill Duff Medical Building, 3775 University Street, Montreal, Quebec, Canada H3A 2B4. E-mail address: silvia.vidal@mcgill.ca

[4] Current address: Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Babraham Research Campus, Cambridge CB2 4AT, United Kingdom.

[5] Abbreviations used in this paper: MCMV, mouse cytomegalovirus; LOD, logarithm of odds; NKC, NK gene complex; ORF, open reading frame; SNP, single nucleotide polymorphism.

0022-1767/06/$02.00

express m157 variants that do not trigger NK cell activation (25), and even resistant B6 mice become highly permissive to viral replication when infected with a mutant MCMV strain that lacks m157 (25, 26). Additionally, the function of other NKC-encoded activating receptors such as NKG2D also appears to have been subverted by MCMV. NKG2D binds at least seven different MHC class I-like cell surface glycoproteins encoded by genes clustered on mouse chromosome 10 (27, 28). Infection of cells with MCMV induces the transcription of mouse NKG2D ligand genes; however, MCMV has evolved three viral genes (*m152*, *m155*, and *m145*) that prevent the expression of NKG2D ligands on the surface of infected cells from classical inbred mouse strains (29–32).

We reasoned that novel mechanisms of MCMV resistance might be found in strains of mice derived from wild specimens of the genus *Mus* that were recently trapped in different geographical locations. These mice are now kept as fully inbred strains and are amenable to genetic studies. Indeed, by crossing them with laboratory strains, offspring can be produced that carry polymorphisms not available in classical inbred strains (33, 34). We have quantified antiviral innate responses in six stocks of inbred mice derived from wild *Mus musculus* specimens and found one resistant to MCMV. Viral titers in spleen and liver were indistinguishable from B6 mice. The new innate resistance mechanism was dependent on NK cells genetically linked to NKC and distinct from *Ly49h*, *Ly49p*, and *Nkg2d*.

Materials and Methods

Mice

C57BL/6 (B6) and BALB/c strains were purchased from The Jackson Laboratory. PWK/Pas (henceforth referred to as PWK), MAI/Pas, MBT/Pas, CAST/Ei, WLA/Pas, WMP/Pas, STF/Pas, and SEG/Pas originate from specimens of wild mice and were maintained at the Institute Pasteur, Paris, France. PWK originates from a specimen of the *Mus musculus musculus* species derived from wild mice trapped near Prague, Czech Republic in 1974. MAI and MBT belong also to the *M. m. musculus* species, whereas CAST belongs to the *Mus musculus castaneus* species and WMP and WLA to the *Mus musculus domesticus* species. The mode of inheritance of the PWK resistance trait was studied in F_1 and F_2 offspring issued from crosses between MCMV-resistant PWK and MCMV-susceptible BALB/c mice. Intercrosses of F_1 mice have failed to produce F_2 mice, most probably due to the Haldane's rule that predicts infertility in the heterogametic offspring of hybrid species (33). However, crosses of F_1 females with parental BALB/c males were fertile and, therefore, were used to produce 75 [(BALB/c × PWK)F_1 × BALB/c] segregating backcross N_2 mice. Five of the 75 N_2 mice were excluded from the analysis because viral loads in the liver were unusually low (<2.5 $\log_{10}$ PFU), likely reflecting technical problems with the infection procedure. Animals were kept at the Central Animal Facilities of the Institut Pasteur and used for experiments at 6–12 weeks of age. All protocols for animal experiments conducted at the Institut Pasteur were reviewed by the Central Animal Facilities of the Institut Pasteur and were done in accordance with guidelines approved by the French Ministry of Agriculture. Some experiments were conducted at the Central Animal Facility of the Medical Faculty of Rijeka, Croatia. Animal work done at the Medical Faculty of Rijeka, Croatia was approved by the local ethical committee and done in agreement with the local regulations.

MCMV infection and plaque-forming assay

The wild-type Smith strain of MCMV was obtained from the American Type Culture Collection and propagated by salivary gland passages as previously described (35) or grown in tissue culture (26). The tissue culture-grown viruses Δm157 MCMV, which lacks open reading frame (ORF) m157, and Δ6 MCMV, which contains a deletion spanning ORFs 144–158, have been previously described by us (26, 29). At 7 wk of age, mice were infected i.p. with 5×10^3 MCMV PFU of salivary gland passaged or 5×10^5 PFU of tissue culture-grown virus. The degree of infection was assessed by determining the number of MCMV PFU in the spleen and the liver 3–4 days postinfection by plaque assay in BALB/c mouse embryonic fibroblasts as described (35). Viral titers were expressed as $\log_{10}$ MCMV PFU per organ. In some experiments, depleting anti-asialo-GM1 or blocking anti-NKG2D (clone CX5 (36), provided by Dr. L. L. Lanier (University of California, San Francisco, CA) mAbs were injected i.p. 2 days before infection.

Flow cytometry analysis

Single cell suspensions of splenocytes were prepared as described (37). Cells were stained with mAbs specific for DX5, Ly49D, Ly49G/I, 2B4, CD3, CD5, CD16, CD69, CD94, CD122, NKG2A/C/E (BD Pharmingen), NKG2D (see previous paragraph), and Ly49H/C/I (clone 1F8 (21), provided by Dr. M. Bennett, University of Texas Southwestern Medical Center, Dallas, TX). In some experiments we used the polyclonal rabbit anti-Ly49H Ab (38), which was detected by a PE-conjugated donkey anti-rabbit IgG (The Jackson Laboratory). For Ly49H intracellular staining, IL-2 activated splenocytes were used in order to have a larger number of NK cells. Although IL-2 induces changes in the expression profile of certain NK cell receptors, it does not perturb significantly the Ly49 receptor repertoire. Cells were analyzed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences). (BALB/c × PWK)F_1 fibroblasts were mock treated or infected with either wild-type MCMV or Δ6 MCMV (1 PFU/cell). Twelve hours postinfection, fibroblasts were analyzed for expression of NKG2D ligands using NKG2D-PE tetramers (28) (provided by D. H. Busch, Technische Universität München, Munich, Germany) as previously described (28).

Cytotoxicity assay

A standard 4-h ^{51}Cr release assay was used to measure NK activity in vitro. Target cells (YAC-1, Ba/F3, and m157-transfected Ba/F3 (22), the latter a gift from Dr. L. L. Lanier (University of California), were labeled with 100 μCi of ^{51}Cr (ICN Pharmaceuticals). Red cell-depleted splenocytes were either used freshly explanted or after culture in RPMI 1640 supplemented with 10% FCS, 5×10^{-5} M 2-ME, 100 μg/ml streptomycin, 100 U/ml penicillin, and 1000 U/ml human IL-2 (R&D Systems) for 5–8 days. Immediately before the assay, cells were stained with anti-DX5, anti-CD122, and anti-CD3 mAb to quantify the number of effector cells that was adjusted so as to have equivalent counts of $CD122^+$ $DX5^+CD3^-$ NK cells in all of the samples of the assay.

Haplotype analysis

We conducted haplotype mapping on genomic DNA using a set of 20 polymorphic markers, including 14 that were previously localized to the minimal genetic interval of *Cmv1* (20, 23, 38, 39). In addition, we used six new markers (*SV175*, *Ly49h(15R)*, *SV50*, *SV151*, *SV168*, and *SV169*) derived from the *Ly49h* genomic DNA sequence (40). Molecular characteristics of these markers are presented in Table I. PCRs were performed using 20 ng of genomic DNA in a 20-μl volume reaction containing 10 pmol of each primer, 0.2 U of *Taq* DNA polymerase (Boehringer Mannheim), and 100 nM dNTPs under previously described conditions (20, 23, 38, 39). Simple sequence and restriction fragment length polymorphisms were visualized by ethidium bromide staining following electrophoresis in 0.5× Tris-borate-EDTA buffer on either 1% regular agarose or 7% acrylamide gels. Products obtained with markers within the *Ly49h* gene, *SV175*, *Ly49h(15/R)*, and *SV50*, were sequenced to confirm their identity in individual mouse strains.

Genotyping and statistical analysis

Genomic DNA was extracted from 70 [(BALB/c × PWK)F_1 × BALB/c] mice tail tips as described (35). Genotypes in the NKC and *H-2* region were

Table I. *Genetic markers*

Locus	Primers[a]	Primer Sequence (5′–3′)	Product Size (bp)
Ly49h	SV175-F	ATGCTCTCCCAATAAAGTTGTTA	290
	SV175-R	TGTACAAGAGATCAGGAAATTGAG	
Ly49h	Ly49h-F	GTTCAAAATTTACTAGTTGC	348
	Ly49h-R	TGTCAAGATAGATAGGAGAGG	
Ly49h	SV50-F	GGAAGGTATTTCCATATTGG	236
	SV50-R	TTACAATTACAGACGATATT	
Ly49h	SV151-F	GTGCTACCACTGAAAACCATTG	204
	SV151-R	CTGTCTCTTGAGTCACCTGCAC	
Ly49h	SV168-F	TTAGCAGAGATAAGTATGCAAGGA	195
	SV168-R	TTTTCTAAGTGTGTAGGTGTGTGG	
Ly49h	SV169-F	CAACATATAAAGAAACAGGACTTG	318
	SV169-R	AAGTGGTCTCAGTGTCTCAGTGT	

[a] F, forward primer; R, reverse primer.

determined by PCR-RFLP. The NKC was amplified using a *Ly49e* marker followed by *Pst*I enzyme digest, as previously described (15). The *H-2* locus was genotyped with the MHC class II-specific primers for *IAA1*. Digestions with both *Hind*III and *Pst*I enzymes allowed the discrimination between the $H\text{-}2^d$ (BALB/c) or $H\text{-}2^b$ (PWK) alleles (41). The contribution of PWK alleles at *NKC* and *H-2* to the segregation of the phenotype (i.e., the $\log_{10}$ of the number of PFU in the spleen) in [(BALB/c × PWK)F_1 × BALB/c] backcross mice was estimated using the following linear model: phenotype = $m + nkc + h\text{-}2 + e$, where *nkc* and *h-2′* are used to represent the number of PWK alleles at each locus, *m* is the common mean value, and *e* represents the usual independent, normally distributed, random deviations. Logarithm of odds (LOD) scores for linkage were calculated by taking the $\log_{10}$ of the likelihood ratio of the model.

cDNA cloning

Total RNA from PWK NK cells was isolated with TRIzol reagent (Invitrogen Life Technologies) and reverse transcribed using SuperScript II polymerase (Invitrogen Life Technologies) with oligo(dT) primers. NK cell receptor cDNAs were amplified with gene-specific oligonucleotide primers for *Ly49s* and *Nkg2d*. Oligonucleotide sequences are presented in Table II. Amplified products were analyzed by gel fractionation, purified with the QIAEX II gel extraction kit (Qiagen), and directly ligated into the pGEM-T Easy vector (Promega). A minimum of three identical clones from two independent PCRs were sequenced for each of the PWK novel genes. DNA and predicted amino acid sequence analysis of these clones was performed using standard nucleotide-nucleotide BLAST (blastn) and standard protein-protein BLAST (blastp) found on the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). The alignment program Clustal W was used for multiple sequence alignments (www.ebi.ac.uk/clustalw).

Results

NK cells confer resistance to MCMV in wild-derived PWK mice

We have measured early immune responses to MCMV in six wild-derived inbred strains of mice derived from the *M. musculus* species. Mice were infected with a sublethal dose of MCMV that readily distinguishes resistant B6 mice from susceptible BALB/c mice at the level of spleen viral titers, but less so in the liver (8, 9) (Fig. 1). Five of six *M. musculus*-derived strains presented high viral titers in the spleen and liver, comparable to those observed in the susceptible strain BALB/c ($\log_{10}$ PFU was 4.0 in the spleen 4.0 and 4.3 in the liver). In contrast, the PWK strain was resistant, showing viral titers of 1.9 $\log_{10}$ PFU in the spleen and 3.8 $\log_{10}$ PFU in the liver, both of which are comparable to titers found in the resistant B6 mouse strain. These results demonstrate that the viral replication pattern in PWK mice is most similar to B6, suggesting that NK cells may be involved.

Further supporting this hypothesis, injection of PWK mice with anti-asialo-GM1, which preferentially depletes NK cells (42), rendered PWK mice relatively susceptible to MCMV infection. Treated PWK mice presented viral loads that were more than two orders of magnitude greater than those of untreated mice (Fig. 2) in both spleen and liver, implicating NK cells in the control of these two organs.

Table II. *Summary of PCR primers used for cDNA amplification*

Locus	Primers[a]	Primer Sequence (5′–3′)	Product Size (bp)
Ly49*h*	Ly49h-F	AGCCTCTTAGGGGATACAGAC	1042
	Ly49h-R	TGTCAAGATAGATAGGAGAGG	
Ly49*k*	Ly49k-F	GATGGGTGAGCAGGAAGTCG	707
	Ly49-k-R	CCACAAATACAGTAGTAGGGAA	
Ly49*n*	Ly49n-F	TTCCCAACTATGAGATTCCAC	770
	Ly49n-R	GCTTTAGATAAAAATAAACATCCTA	
NKG2*d*	NKG2d-F	ACAACCTGGATCAGTTTCTGAAG	779
	NKG2d-R	TCTGGTTGTTGCTGAGATGG	

[a] F, forward primer; R, reverse primer.

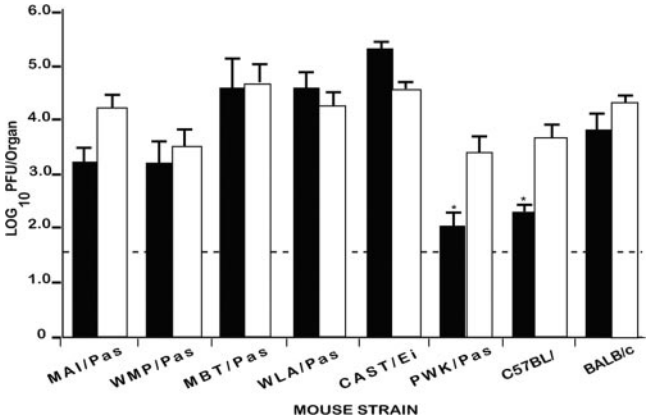

FIGURE 1. Survey of wild-derived mouse strains for MCMV resistance. MCMV titers in the spleen (filled histograms) and in the liver (open histograms) were determined by plaque assay 3 days after i.p. injection of 5×10^3 PFU MCMV (Smith strain, salivary gland preparation). The dashed line indicates the level of detection of our assay ($\log_{10}$ PFU > 1.69). Statistically significant differences in comparison with observed viral titers in MCMV-susceptible BALB/c mice at $p < 0.05$ are indicated by an asterisk (*).

Mode of inheritance of the PWK resistance trait

To study the mode of inheritance of the PWK MCMV resistance trait, we determined the antiviral response in F_1 and N_2 backcross progeny issued from a cross between PWK and MCMV-susceptible BALB/c mice. (BALB/c × PWK)F_1 progeny showed splenic viral loads comparable to or lower than those of resistant PWK and B6 mice (Fig. 3*A*). This finding indicated an autosomal dominant

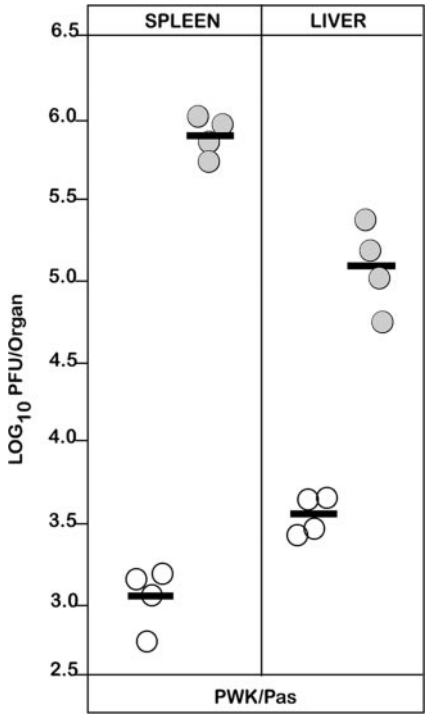

FIGURE 2. Depletion of NK cells abrogates MCMV resistance in PWK mice. Groups of four PWK mice were treated with anti-asialo-GM1 polyclonal Abs two days before MCMV infection (i.p. injection of 5×10^3 PFU MCMV Smith strain, salivary gland preparation). Viral titers from both treated (gray circles) and untreated (open circles) animals were determined in the spleen and liver by plaque assay 3 days postinfection.

mode of inheritance of the MCMV resistance phenotype. Phenotypes of the N_2 progeny presented a bimodal distribution consistent with a major locus effect (Fig. 3*A*). Means of each mode were $\log_{10}$ MCMV PFU 2.2 and 5.8, which are very similar to the values of the MCMV-resistant and MCMV-susceptible parental strains (Fig. 3*A*). We noted, however, that 10% of the N_2 cohort had intermediate values, suggesting that additional genes may influence the phenotype.

Innate resistance to MCMV in PWK mice is genetically linked to the NKC complex

Because our data suggested that NK cells were key players in PWK natural resistance to MCMV (Fig. 2), we hypothesized that a gene in the NKC complex might control the resistance phenotype.

The MCMV-infected N_2 progeny were individually genotyped using the polymorphic NKC marker *Ly49e* and the *H-2* marker *IAA1*. Because the *H-2* locus has been associated with MCMV resistance (15, 43), we decided to include it in our analysis to model the MCMV resistance trait. The statistics supported a two-locus additive model in which both *H-2* and NKC genes play a significant role in the phenotype determination (Table III). The joint LOD score for the model was 25.1 ($p < 2.2e-16$). The proportion of the variation explained by the *H-2* locus was estimated to be 4.4% with a LOD score of 2.7 ($p < 1.9e-4$), whereas that for the NKC was 77.7% with a LOD score of 21.9 ($p < 2.2e-16$). To visualize the effects of the parental alleles, N_2 animals were also separated according to their combined *H-2* and NKC genotypes (Fig. 3*B*). The results clearly demonstrated that PWK alleles at the *NKC* are associated with a 2–3 $\log_{10}$ PFU reduction of viral titers. In contrast, it was also clear that acquiring a PWK allele at *H-2* results in an increase of the mean viral titer by more than one $\log_{10}$ unit, suggesting that the PWK allele at the *H-2* locus is the susceptibility allele.

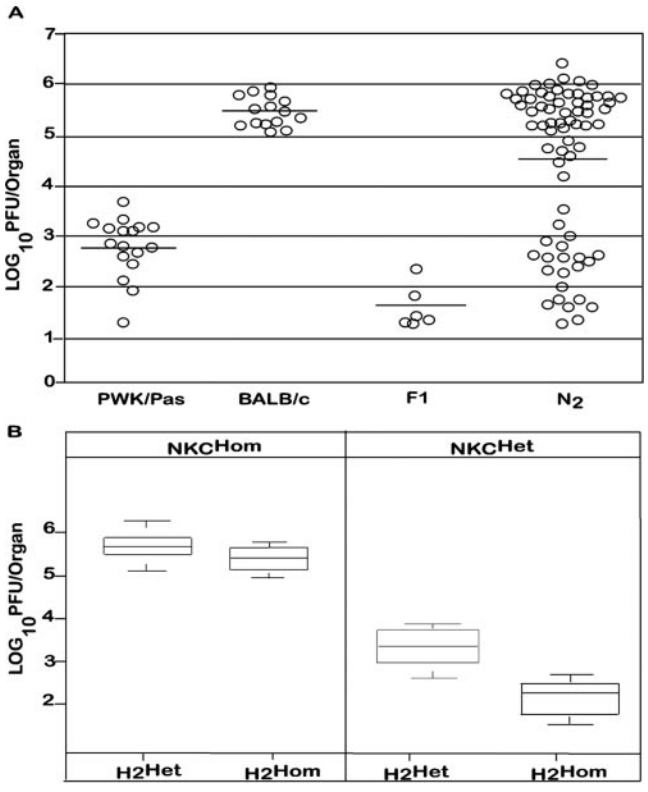

FIGURE 3. Genetic analysis of MCMV resistance in PWK. *A*, Phenotypic distribution of parental PWK ($n = 17$) and BALB/c ($n = 11$) strains and (BALB/c × PWK)F_1 ($n = 6$) and [(BALB/c × PWK) F_1 × BALB/c] N_2 progeny ($n = 70$). Data is viral loads at 3 days after an i.p. injection of 5×10^3 PFU MCMV (Smith strain, salivary gland preparation). Mean is indicated for each group by the horizontal bar across the individual symbols. Each symbol represents an individual mouse. *B*, Combined effects of NKC and *H-2* loci on spleen viral titers. The vast majority of BALB/c homozygous NKC genotypes at *Ly49e* (NKC^{Hom}) were susceptible (40/41). Most heterozygous NKC genotypes at *Ly49e* (NKC^{Het}) were resistant (21/29), yet a bimodal distribution could be appreciated within this group, and 7/29 showed intermediate titers. The two NKC genotypes were plotted against BALB/c homozygous *H-2* genotypes at *IAA1* ($H\text{-}2^{Hom}$) and heterozygous *H-2* genotypes at *IAA1* ($H\text{-}2^{Het}$). The box-and-whisker plot illustrates that, among heterozygous *NKC* genotypes, those that inherited a BALB/c homozygous *H-2* were more resistant, indicating that the BALB/c *H-2* allele is the resistant one.

B6 and PWK may share a common ancestral origin in the Ly49h *region*

The results of the genetic linkage analysis demonstrated that a gene closely linked to *Ly49e* is responsible for resistance to MCMV infection in PWK mice. To explore the existence of other *MCMV* resistance alleles and to study the genotype/phenotype relationship we determined the allelic composition of a set of 41 linked loci in the vicinity of *Cmv1* and studied their haplotypes in a panel of 11 mouse strains, including the six wild-derived inbred strains used in this study plus B6, BALB/c, and 129/J and two additional wild-derived strains belonging to more distantly related *Mus spretus*. We have previously shown that the three latter strains have distinct prototypical haplotypes at the NKC (24, 44). In addition to microsatellites or PCR-RFLP informative markers, we also used six novel markers and 24 single nucleotide polymorphisms (SNPs) overlapping the *Ly49h* gene (Fig. 4). The PWK haplotype presented a unique combination of alleles at the loci analyzed, clearly defining a new NKC haplotype. However, remarkable similarity between PWK and C57BL/6 was observed at the *Ly49h* region with the highest number of SNPs (18/24) conserved between these two strains, indicating a possible ancestral relationship at this region and suggesting a similar MCMV resistance mechanism.

Receptor repertoire in PWK NK cells

PWK NK cells were not stained with mAb specific for NK1.1, CD94, or 2B4 (data not shown). In contrast, they stained positive for NKG2D, NKG2A/C/E, CD69, Ly49C/I, Ly49D, and CD16 and, in line with haplotype results, were also labeled by the 1F8 mAb that detects Ly49H, Ly49C, and Ly49I (Fig. 5, *A* and *B*, and data not shown). Without knowing the sequence of PWK receptors, the possibility that cross-reactivity accounts for positive results could not be excluded. To evaluate the significance of the 1F8 staining, we used an alternative strategy aimed at detecting a Ly49H-specific intracytoplasmic epitope. PWK cells showed some reactivity compared with Ly49H-negative BALB/c cells, however, they did not show the bimodal distribution and bright intensity typical of B6 cells (Fig. 5*C*).

Table III. *Effects of quantitative trait loci controlling MCMV infection*

Locus	*p*	LOD Score	Variance (%)
Model	<2.2e-16	25.1	82.1
LY49e	<2.2e-16	21.9	77.1
IAA1 (H-2)	1.90e-04	2.7	4.4

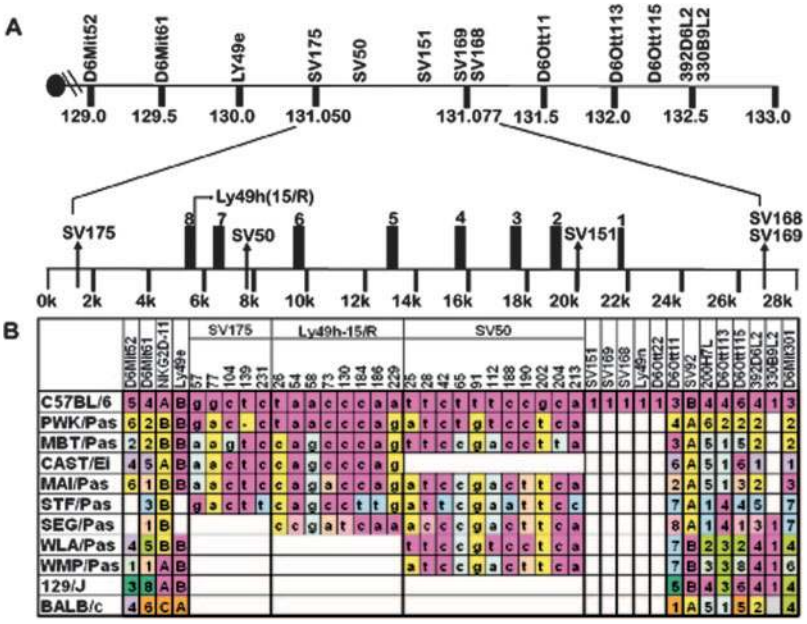

FIGURE 4. Haplotype mapping in the vicinity of *Cmv1* locus. *A*, Physical distance of markers used for haplotype mapping. *B*, Haplotype map of chromosome 6 with 20 polymorphic markers. Numbers indicate PCR product size for microsatellite markers. A color code was assigned to mouse strain-specific alleles (i.e., pink corresponds to B6 and yellow to PWK alleles, respectively). PCR-RFLP markers are distinguishable from microsatellite markers, and the alleles are scored using the letters A and B. SNPs were identified by direct sequencing. SNPs are indicated by their position within the PCR product of origin (SV175, Ly49h(15R), and SV50). Blank squares indicate no PCR product. Individual alleles were arbitrarily color coded, with pink representing B6 alleles.

NKG2D-independent MCMV resistance

As expected from the Ab staining results, cDNA cloning and sequence analysis of the predicted amino acid sequence of the NKG2D receptor from PWK indicated the presence of a functional receptor with only two sequence variants in regard to the B6 sequence in the intracellular domain (H10Y) and the stalk region (I89V), but none in the ligand recognition domain. Thus, one possible explanation for the PWK resistance to MCMV is that PWK-infected cells are somewhat refractory to the immunomodulatory action of MCMV genes on NKG2D ligand expression (29–32). This possibility was ruled out because of the evidence that NKG2D ligands were effectively down-modulated in infected (BALB/c × PWK)F_1 fibroblasts; however, the Δ6 virus containing a deletion spanning ORFs 144–158 is not capable of interfering with the expression of NKG2D ligands (Fig. 6*A*). To validate the biological relevance of our findings, we specifically blocked NKG2D in vivo before infection. The blocking CX5 mAb (36) did not subvert MCMV resistance in PWK mice (Fig. 6*B*). These results ruled out NKG2D activating receptors as candidate for MCMV resistance in PWK mice.

Ly49H and m157-independent MCMV resistance in PWK mice

Although attempts to clone *Ly49h* from PWK NK cells were unsuccessful, we identified three closely related cDNAs coding an expressed pseudogene, *Ly49k*, and full-length *Ly49n1* and *Ly49n2* transcripts highly homologous to *Ly49n* from B6 (Table IV). *Ly49n1 and Ly49n2* lack a highly conserved cysteine residue (position 154) involved in disulfide bond formation, suggesting that these receptors are not functional (Fig. 7). These data also support the idea that the *Ly49* repertoires of PWK and B6 are related but not identical. Although PWK NK cells do not appear to express a bona fide Ly49H, it is possible that an activating receptor in PWK may recognize the viral m157 glycoprotein. A functional cytotoxicity assay was used to test this possibility. Tumor cells of the pre-B cell line Ba/F3 are relatively resistant to NK cell lysis; however, when transfected with m157 they become sensitive to lysis

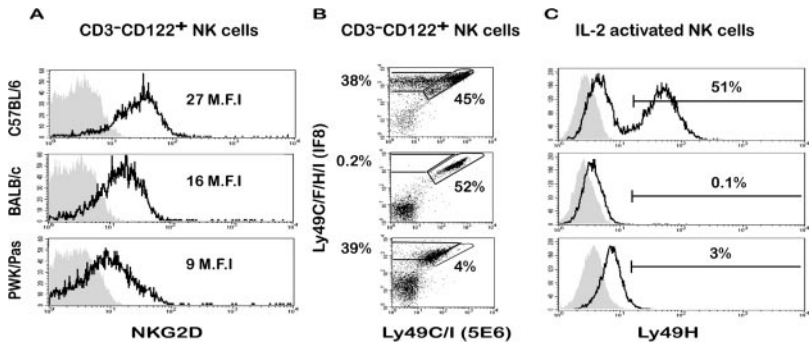

FIGURE 5. Flow cytometry analysis. Cells were obtained from inbred mice B6, BALB/c, and PWK (*top*, *middle*, and *bottom panels*, respectively). *A*, Fresh splenocytes were stained with mAbs specific for CD3, CD122, and NKG2D. CD3-CD122$^+$ NK cells were electronically gated. The histogram shows NKG2D staining against a negative control on gated NK cells. Numbers in histograms represent mean fluorescence intensity. *B*, Fresh splenocytes were stained with mAbs specific for CD3, CD122, Ly49C/I, and Ly49H/C/I. CD3-CD122$^+$ NK cells were electronically gated. The dot plot shows the populations of gated NK cells stained with 1F8 (anti-Ly49C/F/H/I) and 5E6 (Ly49C/I). Numbers in parentheses indicate the percentage of cells stained positive with 1F8 only or with both 1F8 and 5E6 for the indicated mice. *C*, IL-2-activated NK cells were stained with mAbs specific for CD3 and CD122 and then permeabilized and stained intracellularly with polyclonal Ab specific for Ly49H. CD3-CD122$^+$ NK cells were electronically gated. The histogram shows Ly49H intracellular staining against a negative control on gated NK cells. Numbers in histograms represent percentages of cells staining positive.

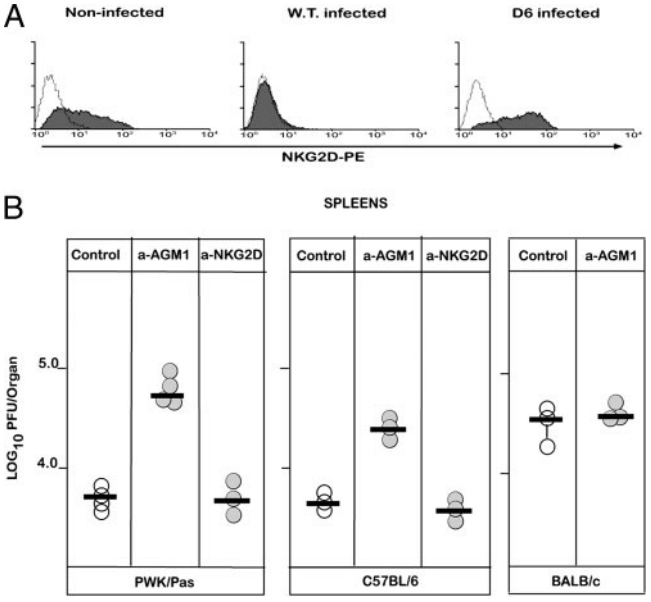

FIGURE 6. Normal down-modulation of NKG2D ligands by MCMV and resistance to all tested mutant MCMVs. *A*, (BALB/c × PWK)F_1 mouse embryonic fibroblasts were infected with the indicated viruses at 1 PFU/cell. Twelve hours later, cells were stained with NKG2D-PE tetramer and analyzed by FACS. In contrast to wild-type (W.T.) virus, mutant Δ6 MCMV failed to down-modulate NKG2D ligands. *B*, Groups of 3–5 mice were injected with the indicated Abs 48 h previously to infection with 5×10^5 PFU MCMV (Smith Strain, tissue-cultured virus). α, anti.

by Ly49H$^+$ NK cells (22). As expected, Ly49H$^+$ B6 cells could readily kill m157-transfected tumor cells. In contrast, PWK and BALB/c NK cells failed to do so, although they did show lytic activity against the prototypic YAC-1 lymphoma targets (Fig. 8*A*, and data not shown).

These results prompted us to test whether PWK mice would still respond to viruses that fail to activate NK cells through m157. Infection with Δm157 MCMV abrogates resistance in B6 mice, because dominant Ly49H-induced responses cannot take place (26). The results in Fig. 8*B* show that PWK mice, contrary to B6 mice, were highly resistant to Δm157 MCMV, providing additional evidence for a new resistance mechanism independent of Ly49H-m157 interactions.

Table IV. *Characterization of Ly49 receptors for PWK*

PWK Receptors	Closest Relative	Identity (%) Nucleic Acid Level	Identity (%) Amino Acid Level
Ly49K (KLRA-psl)	Ly49K^{C57BL6}	88	80
Ly49N1 (KLRA29)	Ly49N/H^{C57BL6}	97	96/95
Ly49N2 (KLRA31)	Ly49N^{C57BL6}	97	98
NKG2D (KLRK1)	NKG2D^{C57BL6}	99	99

Discussion

We have described in this study a new MCMV resistance locus found in the *M. musculus* wild-derived PWK strain. Compared with the reference resistant B6 strain, the PWK resistance pattern showed remarkable similarities and important new aspects. As in B6 mice, viral titers were low in the spleen and higher in the liver early after infection; the resistance was NK cell dependent and genetically linked to the *Ly49* gene cluster at the NKC. Contrary to the B6 strain, however, Ab staining and cDNA cloning indicated the absence of a bona fide Ly49H receptor in PWK mice. PWK mice infected with mutated Δm157 MCMV were resistant to infection, indicating a mechanism of host resistance also independent of m157. This result is not entirely surprising, because a recent study by Voigt et al. (25) has shown that although m157 is crucial for the activation of Ly49H$^+$ NK cells, most wild isolates of MCMV (~86%) present mutations in m157.

We have also shown that the NKC-encoded activating receptor NKG2D was properly expressed in PWK. NKG2D ligands, however, could not be detected in MCMV-infected (BALB/c × PWK)F_1 fibroblasts, indicating that PWK cells were susceptible to evasion strategies adopted by MCMV to escape recognition by NKG2D (29–32). Finally, in vivo blocking experiments of NKG2D did not abolish MCMV resistance in PWK, formally excluding NKG2D as mediator of resistance. Altogether, these observations support the hypothesis that alternative mechanisms other than NKG2D or Ly49H-m157 interactions mediate NK cell dependent resistance to MCMV in the PWK mouse strain.

The NKC haplotypes of the wild strains had different combinations of alleles, but PWK and B6 were similar at the *Ly49h* region, indicating a possible ancestral relationship at this locus. However,

FIGURE 7. Alignment of a novel member of the Ly49h/n/k cluster. The alignment program Clustal W was used for multiple sequence alignments of the predicted amino acid sequences of PWK receptors and Ly49H. Amino acid identities are indicated by a dot, whereas gaps are represented by a dash. Transmembrane and NK cell receptor domains are represented by a dash and solid line, respectively. The arginine residue characteristic of activating receptors is in boldface type. The Ly49N$^{C57BL/6}$ reading frame was restored. The asterisk (*) represents a stop codon naturally found in Ly49N$^{C57BL/6}$ sequence. Cysteines critical for disulfide bond formation are boxed.

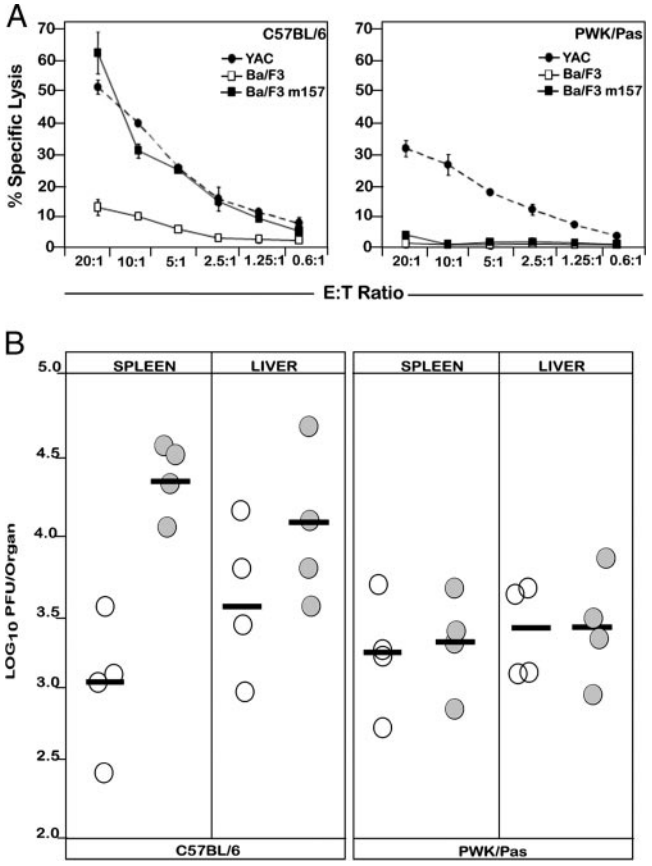

FIGURE 8. m157-independent mechanisms of NK cell immunity to MCMV. *A*, NK cells were purified from B6 or PWK mouse spleens and expanded in vitro in the presence of IL-2. Cytotoxic activity of NK cells was assessed at the indicated E:T ratios in a 4-h ^{51}Cr release assay against prototype YAC-1 target cells or m157-transfected Ba/F3 targets and the control parental Ba/F3 targets. *B*, Groups of 3–4 mice were infected with wild-type MCMV (open circles) or Δm157 MCMV (gray circles). Viral titers were determined in the spleen by plaque assay 3 days after i.p. injection of 5×10^5 PFU of tissue-cultured MCMV viruses.

cDNA cloning indicated the presence of distinct albeit Ly49H-related receptors in PWK, demonstrating the presence of a unique Ly49 receptor repertoire in this strain. A study by Scalzo et al. (45) also demonstrated allelic heterogeneity at NKC loci in populations of free-living *M. m. domesticus* mice, of which only two of 18 specimens were relatively resistant to MCMV. Allelic variability at the NKC among wild-derived strains of mice is not surprising, because high level of variation is a common theme in chromosomal regions containing immune-related genes, deploying the possibility of a wide range of defense options against rapidly evolving pathogens (46–48). The rare occurrence of host resistance against MCMV in wild mice, which are constantly exposed to environmental pathogens, was somewhat unexpected, but the variation at the NKC may also reflect variation in MCMV immunoregulatory proteins (25) and the presence of specific NK cell receptor/ligand pairs occurring during natural infections with MCMV variants. It would be of interest to determine whether infection with wild MCMV isolates, originating from the same geographical location as the wild-derived mouse strains used here, reveals NKC-linked MCMV resistance mechanisms in mouse strains other than PWK.

The dominant NKC gene effect identified in PWK, together with our candidate gene and haplotype analysis, indicate the presence of yet another mechanism of MCMV resistance at a locus, which we named *Cmv4*. Remarkably, viral titers of mice carrying PWK alleles at the NKC and *H-2* were significantly higher than those of mice homozygous at *H-2*, indicating that both *H-2* and *NKC* loci are important for MCMV resistance. The *H-2* effect may reflect a different affinity of PWK NK cell inhibitory receptors for BALB/c or PWK *H-2* gene products, which determine an inhibitory effect on NK cell killing activity against infected cells. Alternatively, the *H-2* effect may reflect an increased affinity of PWK activating receptors for BALB/c *H-2* gene products expressed on MCMV-infected cells, resulting in enhanced NK cell killing activity as has been proposed for the *Cmv3*-mediated resistance in the MA/My model. Our genetic analysis, however, indicated that *H-2* has only a minor contribution to host resistance in PWK in contrast to the NKC gene effect that explains 77% of the variance, suggesting that *Cmv4* operates in a manner similar to that of the *Cmv1/Ly49h* mechanism.

At this point, it is not possible to identify which of the NKC-linked genes, such as *Nkrp*, *Clr*, or other *Nkg2* or *Ly49* gene family members (17, 49), is identical with *Cmv4*. High-resolution linkage mapping and cDNA cloning experiments are warranted to tract down the PWK innate mechanism of host resistance. However, it is tempting to speculate that a novel Ly49 activating receptor that it is directly triggered by a viral product is likely to mediate MCMV resistance in PWK. Smith et al. (23) identified m157 and at least 11 other ORFs encoding molecules with putative MHC class I-like fold. As previously proposed for m157, which binds Ly49H in B6 mice, any other MHC class I-like molecule (for example m144; Ref. 50) could serve as a ligand for an unknown PWK activating receptor signaling target cell killing.

The study of activating receptors and their inheritance in PWK mice will be important for our understanding of the evolutionary role of activating NK receptors and may shed light on human immune resistance mechanisms to infectious diseases. Human killer Ig-like receptors, much like rodent Ly49, show allelic polymorphism (51) and control NK cell functions through conserved mechanisms of intracellular signal transduction (52) despite the structural divergence between Ly49 and killer Ig-like receptors. Human NK receptor genes have been implicated in viral infections (53–55), cell transplantation (56), and pre-eclampsia (57), making this a central topic in modern medicine (51). Mice offer a powerful tool to dissect the genetics of at least some of these associations and to understand the biology of NKC functions.

Acknowledgments

We thank Isabelle Lanctin for skillful help with the wild-derived mouse strains, Seung-Hwan Lee for viral stocks, Michel Bennett, Dirk H. Busch, Martin Messerle, and Lewis L. Lanier for generously sharing reagents, James P. Di Santo for support, and Ute Rogner for critically reading the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Gaytant, M. A., G. I. Rours, E. A. Steegers, J. M. Galama, and B. A. Semmekrot. 2003. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. *Eur. J. Pediatr.* 162: 248–253.
2. Fishman, J. A., and R. H. Rubin. 1998. Infection in organ-transplant recipients. *N. Engl. J. Med.* 338: 1741–1751.
3. Deayton, J. R., C. A. Sabin, M. A. Johnson, V. C. Emery, P. Wilson, and P. D. Griffiths. 2004. Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. *Lancet* 363: 2116–2121.
4. Gandhi, M. K., and R. Khanna. 2004. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infect. Dis.* 4: 725–738
5. Hudson, J. B. 1979. The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infections. *Arch. Virol.* 62: 1–29.
6. Yuhasz, S. A., V. B. Dissette, M. L. Cook, and J. G. Stevens. 1994. Murine cytomegalovirus is present in both chronic active and latent states in persistently infected mice. *Virology* 202: 272–280.

7. Krmpotic, A., I. Bubic, B. Polic, P. Lucin, and S. Jonjic. 2003. Pathogenesis of murine cytomegalovirus infection. *Microbes Infect.* 5: 1263–1277.
8. Allan, J. E., and G. R. Shellam. 1984. Genetic control of murine cytomegalovirus infection: virus titres in resistant and susceptible strains of mice. *Arch. Virol.* 81: 139–150.
9. Scalzo, A. A., N. A. Fitzgerald, A. Simmons, A. B. La Vista, and G. R. Shellam. 1990. Cmv-1, a genetic locus that controls murine cytomegalovirus replication in the spleen. *J. Exp. Med.* 171: 1469–1483.
10. Scalzo, A. A., P. A. Lyons, N. A. Fitzgerald, C. A. Forbes, W. M. Yokoyama, and G. R. Shellam. 1995. Genetic mapping of Cmv1 in the region of mouse chromosome 6 encoding the NK gene complex-associated loci Ly49 and musNKR-P1. *Genomics* 27: 435–441.
11. Beutler, B., K. Crozat, J. A. Koziol, and P. Georgel. 2005. Genetic dissection of innate immunity to infection: the mouse cytomegalovirus model. *Curr. Opin. Immunol.* 17: 36–43.
12. Scalzo, A. A., N. A. Fitzgerald, C. R. Wallace, A. E. Gibbons, Y. C. Smart, R. C. Burton, and G. R. Shellam. 1992. The effect of the Cmv-1 resistance gene, which is linked to the natural killer cell gene complex, is mediated by natural killer cells. *J. Immunol.* 149: 581–589.
13. Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* 17: 189–220.
14. Lodoen, M. B., and L. L. Lanier. 2005. Viral modulation of NK cell immunity. *Nat. Rev. Microbiol.* 3: 59–69.
15. Desrosiers, M. P., A. Kielczewska, J. C. Loredo-Osti, S. G. Adam, A. P. Makrigiannis, S. Lemieux, T. Pham, M. B. Lodoen, K. Morgan, L. L. Lanier, and S. M. Vidal. 2005. Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat. Genet.* 37: 593–599.
16. Brown, M. G., S. Fulmek, K. Matsumoto, R. Cho, P. A. Lyons, E. R. Levy, A. A. Scalzo, and W. M. Yokoyama. 1997. A 2-Mb YAC contig and physical map of the natural killer gene complex on mouse chromosome 6. *Genomics* 42: 16–25.
17. Yokoyama, W. M., and B. F. Plougastel. 2003. Immune functions encoded by the natural killer gene complex. *Nat. Rev. Immunol.* 3: 304–316.
18. Anderson, S. K., J. R. Ortaldo, and D. W. McVicar. 2001. The ever-expanding Ly49 gene family: repertoire and signaling. *Immunol. Rev.* 181: 79–89.
19. Brown, M. G., A. O. Dokun, J. W. Heusel, H. R. Smith, D. L. Beckman, E. A. Blattenberger, C. E. Dubbelde, L. R. Stone, A. A. Scalzo, and W. M. Yokoyama. 2001. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* 292: 934–937.
20. Lee, S. H., S. Girard, D. Macina, M. Busa, A. Zafer, A. Belouchi, P. Gros, and S. M. Vidal. 2001. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat. Genet.* 28: 42–45.
21. Daniels, K. A., G. Devora, W. C. Lai, C. L. O'Donnell, M. Bennett, and R. M. Welsh. 2001. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* 194: 29–44.
22. Arase, H., E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 296: 1323–136.
23. Smith, H. R., J. W. Heusel, I. K. Mehta, S. Kim, B. G. Dorner, O. V. Naidenko, K. Iizuka, H. Furukawa, D. L. Beckman, J. T. Pingel, et al. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* 99: 8826–8831.
24. Lee, S. H., J. Gitas, A. Zafer, P. Lepage, T. J. Hudson, A. Belouchi, and S. M. Vidal. 2001. Haplotype mapping indicates two independent origins for the Cmv1s susceptibility allele to cytomegalovirus infection and refines its localization within the Ly49 cluster. *Immunogenetics* 53: 501–505.
25. Voigt, V., C. A. Forbes, J. N. Tonkin, M. A. Degli-Esposti, H. R. Smith, W. M. Yokoyama, and A. A. Scalzo. 2003. Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells. *Proc. Natl. Acad. Sci. USA* 100: 13483–13488.
26. Bubic, I., M. Wagner, A. Krmpotic, T. Saulig, S. Kim, W. M. Yokoyama, S. Jonjic, and U. H. Koszinowski. 2004. Gain of virulence caused by loss of a gene in murine cytomegalovirus. *J. Virol.* 78: 7536–7544.
27. Cerwenka, A., A. B. Bakker, T. McClanahan, J. Wagner, J. Wu, J. H. Phillips, and L. L. Lanier. 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 12: 721–727.
28. Diefenbach, A., A. M. Jamieson, S. D. Liu, N. Shastri, and D. H. Raulet. 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nat. Immunol.* 1: 119–126.
29. Krmpotic, A., D. H. Busch, I. Bubic, F. Gebhardt, H. Hengel, M. Hasan, A. A. Scalzo, U. H. Koszinowski, and S. Jonjic. 2002. MCMV glycoprotein gp40 confers virus resistance to CD8+ T cells and NK cells in vivo. *Nat. Immunol.* 3: 529–535.
30. Lodoen, M., K. Ogasawara, J. A. Hamerman, H. Arase, J. P. Houchins, E. S. Mocarski, and L. L. Lanier. 2003. NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. *J. Exp. Med.* 197: 1245–1253.
31. Lodoen, M. B., G. Abenes, S. Umamoto, J. P. Houchins, F. Liu, and L. L. Lanier. 2004. The cytomegalovirus m155 gene product subverts natural killer cell antiviral protection by disruption of H60-NKG2D interactions. *J. Exp. Med.* 200: 1075–1081.
32. Krmpotic, A., M. Hasan, A. Loewendorf, T. Saulig, A. Halenius, T. Lenac, B. Polic, I. Bubic, A. Kriegeskorte, E. Pernjak-Pugel, et al. 2005. NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145. *J. Exp. Med.* 201: 211–220.
33. Guenet, J. L., and F. Bonhomme. 2003. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet.* 19: 24–31.
34. Cazenave, P. A., P. N. Marche, E. Jouvin-Marche, D. Voegtle, F. Bonhomme, A. Bandeira, and A. Coutinho. 1990. V β 17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the V β 17 region greatly alter T cell receptor specificity. *Cell* 63: 717–728.
35. Depatie, C., E. Muise, P. Lepage, P. Gros, and S. M. Vidal. 1997. High-resolution linkage map in the proximity of the host resistance locus Cmv1. *Genomics* 39: 154–163.
36. Ogasawara, K., J.A. Hamerman, H. Hsin, S. Chikuma, H. Bour-Jordan, T. Chen, T. Pertel, C. Carnaud, J.A. Bluestone, and L.L. Lanier. 2003. Impairment of NK cell function by NKG2D modulation in NOD mice. *Immunity* 18: 41–51.
37. Colucci, F., C. Soudais, E. Rosmaraki, L. Vanes, V. L. Tybulewicz, and J. P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. *J. Immunol.* 162: 2761–275.
38. Lee, S. H., A. Zafer, Y. de Repentigny, R. Kothary, M. L. Tremblay, P. Gros, P. Duplay, J. R. Webb, and S. M. Vidal. 2003. Transgenic expression of the activating natural killer receptor Ly49H confers resistance to cytomegalovirus in genetically susceptible mice. *J. Exp. Med.* 197: 515–526.
39. Depatie, C., S. H. Lee, A. Stafford, P. Avner, A. Belouchi, P. Gros, and S. M. Vidal. 2000. Sequence-ready BAC contig, physical, and transcriptional map of a 2-Mb region overlapping the mouse chromosome 6 host-resistance locus Cmv1. *Genomics* 66: 161–174.
40. Wilhelm, B. T., L. Gagnier, and D. L. Mager. 2002. Sequence analysis of the ly49 cluster in C57BL/6 mice: a rapidly evolving multigene family in the immune system. *Genomics* 80: 646–661.
41. Peng, S. L., and J. Craft. 1996. PCR-RFLP genotyping of murine MHC haplotypes. *BioTechniques* 21: 362, 6–8.
42. Ehl, S., R. Nuesch, T. Tanaka, M. Myasaka, H. Hengartner, and R. Zinkernagel. 1996. A comparison of efficacy and specificity of three NK depleting antibodies. *J. Immunol. Methods* 199: 149–153.
43. Grundy, J. E., J. S. Mackenzie, and N. F. Stanley. 1981. Influence of H-2 and non-H-2 genes on resistance to murine cytomegalovirus infection. *Infect. Immun.* 32: 277–286.
44. Brown, M. G., A. A. Scalzo, L. R. Stone, P. Y. Clark, Y. Du, B. Palanca, and W. M. Yokoyama. 2001. Natural killer gene complex (Nkc) allelic variability in inbred mice: evidence for Nkc haplotypes. *Immunogenetics* 53: 584–591.
45. Scalzo, A. A., M. Manzur, C. A. Forbes, M. G. Brown, and G. R. Shellam. 2005. NK gene complex haplotype variability and host resistance alleles to murine cytomegalovirus in wild mouse populations. *Immunol. Cell Biol.* 83: 144–149.
46. Khakoo, S. I., R. Rajalingam, B. P. Shum, K. Weidenbach, L. Flodin, D. G. Muir, F. Canavez, S. L. Cooper, N. M. Valiante, L. L. Lanier, and P. Parham. 2000. Rapid evolution of NK cell receptor systems demonstrated by comparison of chimpanzees and humans. *Immunity* 12: 687–698.
47. Mashimo, T., M. Lucas, D. Simon-Chazottes, M. P. Frenkiel, X. Montagutelli, P. E. Ceccaldi, V. Deubel, J. L. Guenet, and P. Despres. 2002. A nonsense mutation in the gene encoding 2′-5′-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. *Proc. Natl. Acad. Sci. USA* 99: 11311–11316.
48. Trowsdale, J., and P. Parham. 2004. Mini-review: defense strategies and immunity-related genes. *Eur. J. Immunol.* 34: 7–17.
49. Iizuka, K., O. V. Naidenko, B. F. Plougastel, D. H. Fremont, and W. M. Yokoyama. 2003. Genetically linked C-type lectin-related ligands for the NKRP1 family of natural killer cell receptors. *Nat. Immunol.* 4: 801–807.
50. Farrell, H. E., H. Vally, D. M. Lynch, P. Fleming, G. R. Shellam, A. A. Scalzo, and N. J. Davis-Poynter. 1997. Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue in vivo. *Nature* 386: 510–514.
51. Parham, P. 2005. MHC class I molecules and KIRs in human history, health and survival. *Nat. Rev. Immunol.* 5: 201–214.
52. Colucci, F., J. P. Di Santo, and P. J. Leibson. 2002. Natural killer cell activation in mice and men: different triggers for similar weapons? *Nat. Immunol.* 3: 807–813.
53. Martin, M. P., X. Gao, J. H. Lee, G. W. Nelson, R. Detels, J. J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, J. Trowsdale, et al. 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* 31: 429–434.
54. Khakoo, S. I., C. L. Thio, M. P. Martin, C. R. Brooks, X. Gao, J. Astemborski, J. Cheng, J. J. Goedert, D. Vlahov, M. Hilgartner, et al. 2004. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 305: 872–874.
55. Guma, M., A. Angulo, C. Vilches, N. Gomez-Lozano, N. Malats, and M. Lopez-Botet. 2004. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* 104: 3664–3671.
56. Ruggeri, L., M. Capanni, E. Urbani, K. Perruccio, W. D. Shlomchik, A. Tosti, S. Posati, D. Rogaia, F. Frassoni, F. Aversa, et al. 2002. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 295: 2097–2100.
57. Hiby, S. E., J. J. Walker, M. O'Shaughnessy K, C. W. Redman, M. Carrington, J. Trowsdale, and A. Moffett. 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* 200: 957–965.