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GENETIC RESISTANCE TO CORONAVIRUS INFECTION

A Review

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GENETIC RESISTANCE TO CORONAVIRUS INFECTION

Introduction

Much information on the mechanisms of host genetic resistance to viral infections has come from research on the coronaviruses, particularly on the mouse hepatitis viruses (MHV). One of the fundamental observations made by Bang and co-workers some thirty years ago was that MHV infection of the host proceeds in a series of stages, which can be seen as three sequential barriers of host resistance[1-3]. These stages have also become the key to dissecting the genetic control of host resistance to coronaviruses. The first stage is the presence or absence of a specific cellular receptor which controls viral entry. Once the virus has gained entry, factors expressed by the host cells will then restrict or permit viral growth and acute disease. Finally, the humoral and cellular defenses of the host's immune system will determine whether the virus is eliminated or disseminated and a chronic disease is established. In this chapter, we have organized our review of genetic resistance to coronaviruses according to these three host resistance mechanisms: genetic control at the level of cellular receptors, genetic control at the level of the macrophage, and genetic control at the level of acquired immunity. However, we would like to stress that these 'levels' are purely operational boundaries. In reality, a host can be infected with a virus several times during its lifetime, and thus all available innate and immune resistance mechanisms will be called into play at once. In addition, we have included a general outline of the methods used to identify host resistance genes in mouse models of infection. Those interested in a more complete explanation of genetic analysis can refer to recent articles on this subject[4,5].

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

Genetic Analysis of Host Resistance to Infections- Brief Outline

Host genetic resistance to viral infections, as for bacterial and parasitic infections, is usually expressed as a complex genetic trait. The initial approach to mapping, cloning and determining the function of the genes regulating resistance to infections is to dissect complex traits, such as disease susceptibility into simpler phenotypes, such as viral replication, that may be under single gene control[5]. The basic procedure is first to develop an animal model of infection, usually in the mouse, that has a clearly defined trait of resistance and susceptibility. Next, the genetic variation of the selected trait is analysed in a large panel of inbred strains. A pattern of resistant, susceptible and intermediate phenotypes (continuous variation) is suggestive of a complex trait controlled by multiple genes, whereas a pattern of clearly delineated susceptibility or resistance (discontinuous variation) suggests a single locus with two alternative alleles[6]. A Mendelian analysis is then undertaken on F1 and segregating backcross populations derived from resistant and susceptible progenitors to determine the mode of inheritance and to give an estimate of the number of genes involved[6]. Should the results indicate that more than one gene is acting then further genetic investigation may require the use of recombinant congenic strains[7] or multiple-locus linkage analysis[6,8]. Should single gene control be confirmed, one of the most frequently used gene mapping methods is linkage analysis in recombinant inbred strains of mice (RIS). The chromosomal location of the unknown locus is deduced by the concordance in the strain distribution pattern in the RIS panel with markers for previously mapped genes. Once the chromosomal location of the pertinent gene is known, gene cloning may be undertaken by positional cloning and/or by the candidate gene approach[4,5].

RESISTANCE TO CORONAVIRUS EXPRESSED AT THE LEVEL OF CELLULAR RECEPTORS

MHV-JHM and MHV-A59

Three classical genetic studies represent the major source of knowledge regarding the genetic control of infection with the neurotropic JHM strain of MHV which causes an acute fatal encephalitis in susceptible mice, and the sero-related, hepatotropic strain MHV-A59 which is considerably less virulent. Stohlman and Frelinger analysed the genetic control of acute encephalitis following JHM inoculation in susceptible B10.S and resistant SJL mice[9]. Their results from backcross and F2 generations supported the hypothesis that resistance to acute disease is under the control of two (unmapped) genes; one, termed *Rhv-1* is dominant and the second, *rhv-2*, is recessive. The genetic analysis also excluded any major effect of the H-2 complex on the resistance to acute JHM-induced disease. Secondly, Knobler et al. analyzed the genetic control of resistance to JHM virus in vivo in resistant SJL and susceptible BALB/C mice[10-12]. They concluded that a single recessive locus termed *Hv2* on chromosome 7, near *Svp-2* (seminal vesicle protein), determined resistance in vivo and in explanted macrophages. In contrast to later studies, these authors concluded that resistance to JHM infection was expressed by macrophages as an ability to restrict viral spread. Finally, Smith and co workers studied the ability of peritoneal macrophages from resistant SJL and susceptible mouse strains to support the growth of MHV- A59 in vitro[13]. They identified and mapped a locus, called *Mhv-1*, to chromosome 7, 41.5 cM from the albino locus, and showed that resistance was inherited in a recessive fashion. However, this group found that resistance was most likely expressed at the level of the viral receptor on the macrophage. Several common threads run through these genetic studies. First, *rhv-2*, *Hv2* and *Mhv-1* are most

likely identical genes, as at least the latter two genes were shown to map to chromosome 7. Secondly, while the exact mechanism(s) of resistance to MHV-JHM and A59 expressed by SJL macrophages is unclear, it seems fair to say that it can be expressed both at the level of the viral receptor and at the level of viral synthesis. Discrepancies between the different studies could have arisen from different doses of virus used, the traits analysed and the treatments employed to obtain peritoneal macrophages. However, the two-gene hypothesis offered by Stohlman and Frelinger may eventually be shown to be correct when later results concerning viral binding to the MHV receptor (CEA/Bgp family of glycoproteins) on SJL tissues are considered.

The receptor for MHV-A59 (termed MHVR) was originally identified by a virus overlay protein blot assay for virus-binding activity as a 110- to 120-kDa glycoprotein on plasma membranes of intestinal epithelium or liver from susceptible BALB/c mice[14,15]. Interestingly, virus binding activity was only detected in membranes from hepatocytes or enterocytes of susceptible BALB/c and semisusceptible C3H mice, but not in comparable preparations of resistant SJL/J mouse membranes[14]. However, membrane preparations from MHV-resistant SJL/J mice were shown to express a homologue of MHVR in studies with antibodies directed against MHVR[15]. Therefore, it was reasoned that SJL/J mice may be resistant to MHV-A59 infection because they lack a functional virus receptor, a hypothesis which is now rejected with the cloning and expression of the MHVR isoforms (see below). Using the monoclonal antibody CC1 which recognizes the N-terminal 25-amino acid sequence of immunoaffinity-purified MHVR, Holmes and colleagues later demonstrated that the MHV receptor was identical to the predicted mature N termini of two mouse genes related to human carcinoembryonic antigen (CEA) and was strongly homologous to the N termini of members of the CEA family in humans and rats[16,17].

Subsequently, several variants of the MHV receptor (MHVR) have been cloned and sequenced[18,19]. MHVR1 is closely related to the murine CEA-related clone mmCGM1 (Mus musculus carcinoembryonic antigen gene family member). The cDNA sequence of this clone can encode a 424-amino-acid glycoprotein with four immunoglobulin like domains, a transmembrane domain, and a short intracytoplasmic tail. A second receptor, $mmCGM_2$, contains two immunoglobulin like domains and encodes a glycoprotein of 42kDa[20]. Additionally, Holmes and coworkers isolated two splice variants of MHVR, one containing two immunoglobulin-like domains [MHVR(2d)] and the other with four domains as in MHVR but with a longer cytoplasmic domain [MHVR(4d)L][18]. Alternative splicing mechanisms could explain how these CEA transcripts are derived from the same gene. All these variants have been recently identified as members of the biliary glycoprotein (Bgp) subfamily of the CEA family[21]. Somatic cell hybrid analysis suggests that the *Bgp* gene is located on chromosome 7 in the mouse[22].

The question of how all the different different MHVR isoforms are related to genetic susceptibility to MHV-A59 infection was then approached by asking whether variants isolated from resistant versus susceptible mouse strains could function as receptors for MHV-A59[18,19,23]. Phenotypically susceptible inbred mouse strains BALB/c, C3H, and C57BL/6 were found to express transcripts and proteins of the MHVR1 ($mmCGM_1$) isoform and/or its splice variants but not the $mmCGM_2$ isoform. In contrast, adult SJL/J mice, which are resistant to infection with MHV-A59, express transcripts and proteins only of the mmCGM2-related isoforms, not MHVR[18]. This strain distribution is compatible with the hypothesis that the MHVR and $mmCGM_2$ glycoproteins may be encoded by different alleles of the same gene. Surprisingly, especially in view of the structural differences between MHVR and $mmCGM_2$, both of the groups of Holmes and Lai have shown that transfection and expression of either $mmCGM_1$ or $mmCGM_2$ from SJL mice into MHV-resistant Cos 7 or BHK cells rendered the cells susceptible to MHV infection[18,19]. The ability of the SJL-related isoforms molecules to serve as MHV receptors was comparable to that of those from

C57BL/6. Thus, another factor must explain the genetic resistance of the SJL mouse in spite of its functional MHV receptor. Several possibilities have been put forward such as post-translational modification, but the most intriguing idea brought out by Yokomori and Lai[19], and one that fits well with the earlier two-gene hypothesis of Stohlman and Frelinger[9], is that the mechanism of genetic resistance of the SJL mouse lies in the product of a second gene whose product may be associated with the MHV receptor. This second factor of resistance has been envisioned as a protease that acts very early during virus entry to interfere with other step(s) in virus replication.

Cellular Receptor for Human 229E and Pig TGEV Coronaviruses

A second receptor, aminopeptidase N (APN), which in humans is identical with the CD13 differentiation antigen[24], has been identified as a receptor for both human and pig-specific coronaviruses[25,26]. However, genetic control in this case has only been demonstrated in humans[27]. Yeager et al. showed that human APN, located on chromosome 15[28] is a receptor for one strain of human coronavirus 229E, that is an important cause of upper respiratory tract infections[26]. A study of somatic cell hybrids demonstrated that a gene for susceptibility to the coronavirus 229E is located on chromosome 15 in the region q11-q12[27]. Secondly, APN has been identified as a receptor for transmissible gastroenteritis virus (TGEV), a coronavirus which causes fatal diarrhea in the newborn pig[25]. TGEV replicates selectively in the differentiated enterocytes covering the villi of the small intestine. In the small intestine, APN plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. Two lines of evidence supported the view that APN itself acts as a receptor. First, virions bound specifically to APN that was purified to homogeneity. Second, transfection of the APN gene into an otherwise non-permissive cell line conferred susceptibility to TGEV.

APN is thought to be involved in the metabolism of regulatory peptides by diverse cell types, including macrophages, granulocytes, and synaptic membranes of the central nervous system (CNS). In the mouse, the APN enzyme has not been identified as a coronavirus receptor, but the *Lap-1* locus, (for leucine arylaminopeptidase-1 also called APN) has been linked to chromosome 9 in recombinant inbred lines and in intraspecific backcrosses[29]. Interestingly, mouse APN has also been identified in antigen-presenting cells and is co-expressed with H-2 class II molecules[30]. It is not known if the strain distribution for *Lap-1* would fit any genetic model for MHV, for example the enteric strain MHV-Y, but it may be worth considering as a candidate gene/receptor especially considering that APN is expressed in macrophages.

GENETIC RESISTANCE TO MHV EXPRESSED BY MACROPHAGES

The MHV virulent type 2 (MHV-2) and 3 (MHV-3) strains induce acute hepatic failure in susceptible mouse strains following intraperitoneal injection. Death from MHV-2 infection occurs 2-3 days after inoculation and probably results from the destruction of hepatic parenchyma and the formation of large necrotic foci[1]. In acute MHV-3 infection, susceptible mice die within 5 days with the occurrence of hepatic necrosis, fibrin deposition and a heavy infiltrate of inflammatory cells[31]. Resistance to acute infection with MHV-2 and MHV-3 has been shown to be dependent on innate macrophage factors[1,2,31-33] as well as immune defense mechansims[32,34,35] rather than

on cellular receptors. Below, we discuss the macrophage resistance mechanisms described in acute MHV-2 and MHV-3 infections which appear to be controlled by different, H-2-unlinked genes.

MHV-2

The *Hv1* locus, described by Bang and coworkers in the 1960's, controls susceptibility to lethal infection by MHV-2[1-3]. In this model, the dominant, susceptible allele of *Hv1* occurs in the PRI (Princeton) strain and the C3HSS congenic strain whereas the resistant, recessive *Hv1* allele occurs in the C3H strain. Resistance or susceptibility in vivo was shown to correlate almost perfectly with the permissivity of peritoneal macrophages to MHV-2 in vitro. The *Hv1* gene is probably not expressed as a viral receptor, since virus was shown to be equally well adsorbed by both resistant and susceptible macrophages[2]. Resistant macrophages were shown to block some aspect of viral RNA synthesis, thus affecting viral replication. Moreover, genetically susceptible mice could be rendered phenotypically resistant by treatment with the lectin concanavalin A (Con A), similarly, macrophages harvested from the Con A treated animals also manifested resistance in vitro[3]. Con A had no effect when administered directly to the macrophages, suggesting that reversal of susceptibility occurred following the induction of lymphokines. The chromosomal location of *Hv1* is unknown, but it segregates independently of the *Hv2* locus on chromosome 7, the *Bcg* locus on chromosome 1 and the locus for resistance to flavivirus on chromosome 5[36].

MHV-3

Genetic resistance to acute MHV-3 infection is comprised of at least two different host defense strategies. The acute phase of MHV-3 infection is characterized by a fulminant hepatic necrosis which kills susceptible strains of mice such as C57BL/6 and BALB/c within 3-5 days. During this phase, the host cells for viral replication are both macrophages and lymphoid cells[32], and, consequently, both of these cell types have adapted equally important resistance mechanisms. The group of Levy has extensively studied the role of the macrophage in the resistance to hepatitis caused by MHV-3[31,33,37-39]. Here, the inheritance of the trait of resistance/susceptibility to the fulminant, acute hepatitis and death caused by MHV-3 infection was analyzed in a set of recombinant inbred (RI) strains of mice derived from the resistant A/J and susceptible C57B1/6J progenitors[37]. The strain distribution pattern (SDP) showed a discontinuous variation ranging from fully resistant (no liver disease), to fully susceptible (death from fulminant hepatitis), with 16 RI strains showing intermediate degrees of susceptibility. This SDP was consistent with a two-recessive-gene model of resistance. These results were in contrast to earlier results (1979) of Levy-Leblond et al.[40], who found evidence for a single, recessive gene for resistance to acute MHV-3 disease in a panel of F1, F2 and backcross generation mice. However, this study [40] clearly showed that the gene(s) for resistance express an age-dependent penetrance. No evidence of linkage between acute MHV-3 sensitivity and genes of the H-2 complex was found in either of the two studies. The SDP for susceptibility/resistance to MHV-3 was also found to correlate with the induction of procoagulant activity (PCA), a prothrombinase[37]. PCA mediates inflammatory responses by virtue of its direct prothrombin cleaving activity [38]. The cellular nature of the production of the 74 kDa PCA molecule was also examined in RI strains and a restriction for induction of PCA was observed at the level of the macrophage[39]. Peritoneal macrophages from resistant parental A/J and RI strains could not be induced to express PCA when stimulated by MHV-3 alone or in the presence of lymphocytes from susceptible and H-2 compatible RI mice. In contrast, macrophages from susceptible RI strains of mice expressed a similar increase in PCA after stimulation with MHV-3 in the presence of L3T4$^+$ (T helper cells, CD4$^+$)

lymphocytes. In addition, T cells from MHV-3 immunized resistant RI mice, but not from unimmunized mice, were able to suppress induction of PCA. This suppressor activity could be detected in resistant mice even after 28 days of infection. The requirement for both T helper cells and macrophage PCA production for the full expression of MHV-3-induced acute hepatitis is consistent with the hypothesis of a two-gene model for susceptibility.

Two treatments have been found to inhibit the severe liver disease and procoagulant activity induced by MHV-3. In one, 16,16 dimethyl prostaglandin E2, dmPGE2, has been shown to specifically inhibit the activity of the PCA molecule by a posttranslational mechanism[33]. However, PGE2 treatment did not block MHV-3 viral replication or prevent death in susceptible animals. PCA activity could not be inhibited by other eicosanoids including prostacyclin (PGI2), PGF2a and leukotriene B4 (LTB4). In the second treatment, passive transfer of the anti-PCA monoclonal antibody 3D4.3 attenuated the development of hepatic necrosis and enhanced survival in a dose-dependent manner that correlated with the rates of metabolism of immunoglobulin[31]. Interestingly, the passive transfer treatment, in contrast to the E2 treatment, did decrease replication of MHV-3 in livers of susceptible animals to levels equivalent with those seen in resistant A/J mice. Taking the results of these two treatments together, the authors suggested that PCA has multiple pathogenic roles in MHV-3 infection[31]. First, PCA may activate the coagulation system that enhances hepatic necrosis, and secondly, they speculated that PCA could promote the proteolytic cleavage of the structural spike (S) protein into S1 and S2 subunits, thereby activating the cell fusion properties of the virus[41].

GENETIC RESISTANCE EXPRESSED AT THE LEVEL OF ACQUIRED IMMUNITY

According to our initial scheme, we have now arrived at the last 'level' of genetic resistance: that of acquired immunity to MHV. From a genetic point of view, this is the most difficult phase to analyse. The role of neutralizing antibodies, cellular immunity (including T, B, NK and antigen presenting cells) and the importance of the different antigenic epitopes of the virus are all elements which clamor for attention and surely would require an entire chapter to be discussed adequately. For our review, we have chosen to discuss the relationship between immunodeficiency and MHV-3 disease, as studied by Lamontagne and co workers[34,35,42], and secondly to review studies describing the role of CD4 and CD8 T cells during chronic JHM infection[43,44].

MHV-3

During the acute phase of MHV-3 infection, the role of immune defense mechanisms was initially revealed by evidence that resistant A/J mice become susceptible to the hepatic necrosis following immunosuppressive treatments[45]. More recently, Lamontagne et al have shown in vitro that the pathogenic L2-MHV3 strain could infect macrophages as well as lymphocytes, including thymocytes of both susceptible (C57BL/6) and resistant (A/J) mice[35]. In resistant A/J mice, however, a reduction in the level of infectious viral particles by the infected cell occurred at 48 hrs postinfection. This cellular resistance mechanism was observed to act as a recessive phenotype which acts to prevent viral replication very early following infection. Furthermore, these authors have shown that the pathogenic outcome of MHV-3 disease in susceptible mice correlated with the ability of the virus to cause lysis in vitro of Thy1.2$^+$ and surface IgM$^+$ cells. Susceptible mice also demonstrated significant atrophy of the thymic and splenic lymphoid follicles by 72 hours post infection, whereas no

such atrophy or cell lysis occurred in resistant A/J animals. Moreover, it was determined by a virus interference assay that the virus could attach to the cell surface, and that the resistance mechanism acts to interfere with the activity of the viral RNA polymerase. These findings thus demonstrated that T and B lymphocytes can express intrinsic, H-2 unlinked[40] genetic resistance to the MHV-3 virus.

Lamontagne and co-workers have also begun to explore the consequences of the MHV3-induced immunodeficiency on the chronic phase of the disease[34]. Chronic MHV-3 infection results in aged F1 animals (bred from a cross of resistant A/J and susceptible C57BL/6 mice) who survive the acute hepatitis[40]. The disease is characterized by viral persistency in various organs, including the brain, spleen, and thymus and the occurrence of hindlimb paralysis at approximately 2 months after infection. It was determined that T and B cell depletions arose in F1 animals within a few days after infection, and low levels of splenic T and B cells were maintained for up to 3 months, until death of the mice[34]. The reduction of splenic lymphoid cells was determined to originate from depletion of all T cell subpopulations in the thymus and of pre-B and B cells in the bone marrow. However, infectivity studies performed in vitro revealed that the virus established a non-productive replication in thymic stromal cells with a low level of viral transmission to complexed thymocytes, whereas pre-B and B cells supported a productive, lytic viral replication.

The trait of hindlimb paralysis in the chronic form of MHV-3 disease has been genetically analysed by Levy-Leblond et al[40]. The analysis was performed in F1, F2 and backcross animals as well as in a panel of A strain mice congenic at the H-2 complex. The development of paralysis in the genetic crosses suggested that the chronic disease was controlled by one or two recessive genes. However, when the panel of A strain H-2 congenics was analysed, it appeared that the genetic control was linked to the H-2 complex, since the A.CA strain ($H\text{-}2^f$) was the only one which displayed complete resistance to the chronic disease. Using H-2 recombinant strains of mice, it was later determined that both class I H-2K and H-2D regions controlled the resistance to the development of paralysis[46]. Further analysis of the panel of H-2 recombinants for the trait of T and B cell depletions described above[34] could reveal if and how H-2 genes, or closely linked genes such as tumor necrosis factor (TNF)[47] or the transporter for antigen processing genes (TAP)[48], are involved in the development of MHV-3-induced immunodeficiencies.

MHV-JHM

In the first section of this review, we discussed how resistance to JHM-induced acute encephalitis is controlled by the MHVR (CEA) receptor and a second, unknown resistance factor. There is considerably less information concerning the basis of genetic resistance and susceptibility to the chronic phase of disease induced by the JHM virus, which is considered to be a model of central nervous system (CNS) disease. Susceptibility to the chronic disease is characterized clinically by the development of hindlimb paralysis 3-8 weeks post infection and histologically by evidence of demyelination in infected brains[44,49]. One model of resistance or susceptiblility to the late onset disease is called the maternal antibody protection model where the strains BALB/c and C57BL/6, which are fully susceptible to acute encephalitis, are protected from acute disease when immunized intranasally and nursed by immunized dams[49]. However, from 40-60% of the C57BL/6 mice will go on to develop late onset demyelinating disease whereas BALB/c mice are resistant and do not develop chronic symptoms. Neutralizing antibodies do not protect susceptible mice from the late onset disease, signifying that T cells may have the crucial role in viral clearance and in preventing CNS disease.

Table 1. Mouse loci controlling resistance to Coronavirus MHV infection

Locus	Trait	Chromosome	Inheritance of resistance	Reference
MHV-2				
Hv1	survival/replication in macrophages	?	recessive	1-3
MHV-3				
nd	survival/macrophage procoagulant activity	? [H-2 unlinked]	2 genes, recessive	37
nd	development of chronic disease[paralysis]	17, H-2K, H-2D	1 or 2 genes, recessive	40
MHV-JHM/A59				
Hv2 [? *rhv2*, *Mhv1*, *Bgp*]	infection of macrophages, neurons, liver cells	7	recessive	10-13, 22
Rhv1	survival/acute encephalitis	?	dominant	9
Pj1	chronic disease[paralysis]	7	?recessive	50
nd	chronic disease[paralysis]	17, H-2D^d	?	43,44

nd, not designated

It was determined by Stohlman's group in adoptive transfer experiments that class I H-2D restricted T cells of the helper phenotype (CD4+) and cytotoxic phenotype (CTL; CD8$^+$) were required for viral clearance[43]. This group also detected a strain difference correlating with resistance to viral elimination in that BALB/c mice mounted a CD8$^+$ response against the nucleocapsid (N) protein, whereas C57BL/6 mice did not. Castro et al pursued this question by determining, in H-2 congenic mice, whether replacement of the C57BL/6 type D^b locus by the BALB/c type D^d locus was sufficient to protect mice from the late onset disease[44]. The results showed that B10.A(18R) mice, which contain the d alleles of the D and L loci, exhibited a CTL response against the N protein as did the BALB/c mice, however, approximately 16% of the B10.A(18R) mice developed late onset symptomatic disease. These results suggested that the CD8$^+$, N-specific response is only partially protective against the development of the demyelinating disease. Additional results from this study showed that acutely and chronically JHM-infected C57BL/6 mice manifested an S protein specific CTL response in brains and spinal cords. BALB/c mice, however, did not contain anti-S CTL activity. Thus, the anti-S CTL activity present in the CNS of chronically infected animals could be involved in the pathogenesis of demyelination. Although the relationship of the CD4$^+$/CD8$^+$ interaction to CNS demyelination remains to be determined, it is apparent that several factors governing T cell function are likely to be involved[44].

The involvement of several factors is also supported by the data of Kyuwa et al, who have examined the genetic control of acute and late disease induced by MHV-JHM in the strains BALB/cHeA, STS/A, F1 hybrids and 13 (BALB/cHeA X STS/A) RI strains[50]. In this model, following intracerebral inoculation with JHM, all the BALB/cHeA mice died within 2 weeks from acute encephalitis whereas only 30% of STS/A mice died and were termed semisusceptible. The genetic analysis of the acute phase suggested the involvement of multiple genes, in agreement with the earlier studies of Stohlman and Frelinger[9]. For the chronic disease, the paralysis of delayed onset developed in 36% of STS/A, 40% of the F1 hybrids and eight of the 13 RI strains. However, the incidence varied widely among the RI strains, indicating that JHM-induced late disease is under multifactorial control. An alternative hypothesis is that variability of trait expression is large. This is supported by analogous results in the model of acute sensitivity to MHV-3 infection where results in RI and F1 mice suggested a highly variable, age-dependent

penetrance of the resistance allele(s)[40]. Indeed, by ordering the mouse strains into two disease categories and evaluating their data considering a single gene hypothesis, Kyuwa et al found that the SDP indicated a putative gene effect located on chromosome 7, near Ly-15, which they designated *Pjl*[50]. The authors speculated that since Ly-15 is known to facilitate T cell recognition [51] and to be involved in the CTL response[52] it is possible that Ly-15 is involved in the CTL response during CNS disease. Therefore, the Ly-15 antigen could be a candidate for one of the additional factors governing CTL function in the maternal antibody protection model.

CONCLUDING REMARKS

It is apparent from reviewing these studies that host resistance to infection with MHV is under multigenic control (summarized in Table 1). As we said earlier, although the three levels of host resistance we discussed are artificial boundaries, the advantage of setting such boundaries is that one can functionally dissect a complex trait into parameters that are under single gene control. In this way, genetic susceptibility to MHV-3 acute fulminant hepatitis has already been disassociated into several traits, such as macrophage PCA production and lymphoid cell depletion. Once the responsible genes have been mapped , it may be possible to analyze the genetic control of MHV-3-induced hepatitis (or JHM-induced chronic CNS disease) by the use of multiple linked marker loci[6]. We await with interest as the story of genetic resistance to coronavirus infections continues to unfold.

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