Jean-Louis Guénet
is an emeritus scientist at the Institut Pasteur, where he has spent most of his career as a mouse geneticist. His major contributions concern the development of the mouse genetic map and the development of inbred strains from recently trapped wild specimens. His team was one of the first to identify the mutation in the *Oas1* locus, responsible for susceptibility of mice toward flaviviruses.

Assessing the genetic component of the susceptibility of mice to viral infections

Jean-Louis Guénet

Jean-Louis Guénet
Département de Biologie du Développement,
Institut Pasteur,
75724 Paris CEDEX 15, France

Tel: +33 (0)1 45 68 85 54
Fax: +33 (0)1 45 68 86 34
E-mail: jlguenet@wanadoo.fr

Abstract

Laboratory mice often exhibit wide differences in susceptibility when infected experimentally with viruses. Based on such observations, experiments have been designed to investigate the determinism of these differences at the molecular level, and a few genes that play a major role in the innate mechanisms of defence of the species toward viral aggressions have been characterised. For example, the extraordinary resistance of SJL mice to experimental infections with hepatitis virus strain A59 is the consequence of a structural alteration of a cell adhesion molecule which normally binds to the spikes of the virus, allowing its entry into the cells. If the virus cannot bind to the molecule, or if the molecule is absent, epithelial cells of the intestine and liver are not infected and mice are resistant. In the same way, most — not to say all — laboratory strains of mice are susceptible to infections with orthomyxoviruses or flaviviruses because essential molecules, the synthesis of which is normally triggered by interferon, are defective in these mice. Wild mice, by contrast — probably because they are constantly exposed to natural infections — are resistant. Finally, some mouse strains resist experimental infections by the mouse cytomegalovirus 1 (MCMV-1) because, once infected, these mice synthesise a molecule at the surface of infected cells which allows immediate recognition and killing by natural killer (NK) cells. With the exuberant development of mouse genetics and the constant generation of new mutant alleles, it is likely that many more genes with an impact on the phenotype of resistance or susceptibility will be identified in the forthcoming years. These genes are probably numerous, however, and many of them presumably interact with each other and/or have additive effects. This might slow down progress in our understanding of the innate mechanism of defence.

INTRODUCTION

The severity and outcome of viral infections may be influenced by a number of factors. Some of these factors, for example the age of the host, its physiological status or previous infections with similar infectious agents, are environmental by nature. Other factors, by contrast, depend on the genetic constitution of both the host and the infectious agent, and it is for this reason that the encounters between the two organisms have sometimes been compared with the 'battle' of two genomes.[1] Indeed, co-evolution of viruses with their hosts towards less deleterious infections must be advantageous for both organisms, since any viral disease leading ruthlessly and regularly to the death of the host would also lead to the disappearance of the virus. Such situations may have occurred during evolution but, of course, there are no clues to substantiate this assumption.

In the human species, several observations of inherited resistance (or susceptibility) to specific pathogens have been reported. Resistance to malarial infection was one of the first examples, while resistance to HIV infection is one of the most recent. In both cases, the observation led to the discovery of a

single Mendelian locus: the sickle-cell trait in the first case (HbS), the gene encoding the CC chemokine receptor 5 (CCR5) in the latter.[2,3] There have also been reports of other cases of resistance or susceptibility to infectious microorganisms that were controlled by single Mendelian units. In most instances, however, the observed differences have a more complex determinism, with several genes or quantitative trait loci being involved, reflecting the complexity of the host/virus interactions.[4]

Using the mouse as a model species for investigating the genetic component of susceptibility to viral infections is advantageous because it is possible to test homogeneous populations (inbred strains) using different routes of inoculation and various doses or strains of virus. In addition, once a genetic difference has been observed between any two strains, the genes that are involved in determining this phenotype are amenable to molecular analysis. Finally, another advantage of the mouse species is that any gene that is suspected *a priori* to play a role in the pathophysiology of cellular infection may be modified by *in vitro* genetic engineering for further analysis. For all of these reasons, and despite the fact that viruses that are pathogenic to mice are not always pathogenic to man, it has been possible over the past few decades to identify a number of host cellular genes modifying the susceptibility of mammalian cells to particular viruses.[5–7]

These genes, in general, act at one of three discrete steps of viral infection: 1) when the virus binds to the cell surface or when it enters the cell; 2) during the early phases of replication, interfering with the so-called innate mechanisms of immunity; or 3) during the later phases of virus production. This review will consider the cases in which genetic analysis of inter-strain differences have allowed the identification of discrete loci with a major influence on susceptibility to experimental viral infections, with emphasis on those cases that have been

investigated up to the molecular level by the strategy of forward genetics.

FLAVIVIRUSES

Flaviviruses are positive-sense, single-stranded RNA viruses which are generally transmitted by arthropod bites (mosquitoes or ticks). In most cases, infections caused by these viruses are mild or even unapparent, indicating that some degree of adaptation between the virus and its host has occurred. In some regions, however, mosquito-borne flaviviruses can cause epidemic outbreaks in humans, and infected patients may exhibit a wide range of symptoms, ranging from transient febrile illness to life-threatening haemorrhagic fevers (dengue and yellow fever) or meningo-encephalitis syndromes (Japanese encephalitis and West Nile fever). The reasons why flaviviruses cause severe clinical manifestations only in a small percentage of infected individuals have not yet been elucidated, but recurrent epidemiological observations and recent scientific data indicate that host-dependent genetic factors might be important. Variations in mouse innate flavivirus susceptibility were reported for the first time in the early 1930s. Investigations performed during the following decades indicated that a major locus on chromosome (Chr) 5, designated flavivirus resistance (*Flv*), was responsible for the observed phenotype with, basically, two alleles: Flv^r, which is dominant and induces resistance and Flv^s, which is recessive and correlates with susceptibility. For historical details on the discovery and genetics of the *Flv* locus, refer to Shellam *et al.* (1998) or Brinton and Perelygin (2003).[8,9] Surprisingly, most (not to say all) laboratory-inbred strains of mice are susceptible to experimental infections, while most wild mice are resistant. To mention just one example of this dramatic difference in susceptibility, the author's group reported that a single intraperitoneal inoculation equivalent to 100 LD_{50} of the West Nile (WN) virus (strain IS-98-ST1) administered to adult mice of the classical

Laboratory-inbred strains of mouse are artificial populations stemming from three subspecies of the genus *Mus*. Accordingly, the genome of a given strain is a unique mosaic, with variable proportions of the three components. It is then surprising that all strains are susceptible.

laboratory inbred strains BALB/c and C57BL/6, was lethal for all of these animals 9.5 ± 1.5 days after inoculation, while mice from totally unrelated inbred strains recently derived from wild ancestors of either the *Mus m. domesticus* (WMP/Pas), *Mus m. musculus* (MAI/Pas, MBT/Pas, PWK/Pas) or *Mus spretus* (SEG/Pas, STF/Pas) species, or from laboratory strain PL/J, were resistant to the same treatment.[10] During this experiment, infectious particles of WN virus could be detected in the brains of infected mice after five days of infection, and the amounts of virus peaked at 10^9 focus forming units/g of brain tissue by day 7. High levels of anti-WN antibody could also be detected in surviving animals, indicating that the virus replicated in resistant strains. This study was no more than a recapitulation of the many similar experiments that had been carried out over the previous 40 years, with a variety of flaviviruses using several routes of inoculation and several doses and strains of virus.[8,9] All of these experiments yielded similar results, confirming that the phenotype of resistance/susceptibility is not WN-specific but, on the contrary, extends to other types of flaviviruses as well. It is now established that the phenotype of resistance/susceptibility co-segregates with a point mutation in the gene *Oas1b*, encoding the 1B isoform of $2'$-$5'$ oligoadenylate synthetase (OAS1B).[10,11] Although the *Oas1b* gene exhibits several single nucleotide polymorphisms (SNPs) among the different strains or species of mice, it is remarkable that all susceptible mice tested so far have a $T{\rightarrow}C$ transition that replaces an arginine residue with a premature stop codon in the fourth exon of the gene. The perfect correlation between susceptibility to viral infection and the occurrence of a stop codon supports the hypothesis that a truncated, and presumably inactive, form of $2'$-$5'$ OAS L1 is indeed the cause of the innate susceptibility to flavivirus infection. The presence of a stop codon is also compatible with susceptibility behaving as

a recessive trait and fits perfectly with one of the known functions of the interferon-inducible enzyme $2'$-$5'$ OAS, which is to synthesise $2'$-$5'$ oligoadenylates which in turn activate latent ribonuclease L (RNase L), ultimately resulting in degradation of viral RNA and inhibition of viral replication.[12]

In vitro experiments, performed with stable neuroblastoma cell clones overexpressing either the mutant or wild-type OAS1B, indicated that replication of the WN virus is less efficient in cells that produce the normal copy of OAS1B than in those expressing the mutant form of the protein, reinforcing the idea that the *OAS1B* gene is critical for controlling viral pathogenesis.[13]

The structural organisation of the genes encoding $2'$-$5'$ OASs has been studied in a few species, and in detail in humans and mice. In humans, it is a cluster of three genes designated *OAS2*, *OAS3* and *OAS1*, respectively (Figure 1).[14–18] Human *OAS2* and *OAS3* have mouse orthologues in *Oas2* and *Oas3*, and the transcription products of these genes are also very similar, with two alternatively spliced isoforms encoded in mouse *Oas2*, consistent with the existence of human isoforms p69 and p71 encoded in human *OAS2,* while — similar to the case in humans — there is only one transcript from mouse *Oas3*. The function of the proteins encoded in *OAS2/Oas2* and *OAS3/Oas3* is not yet clearly established. The structural organisation of *OAS1* is very different in humans and in the mouse. In humans, there is only one *OAS1* gene encoding four different OAS proteins (p42, p44, p46 and p48), resulting from an alternative splicing of the first five exons with the three exons of the C-terminal region (Figure 2). In the mouse there are no less than eight transcription units, orthologous with the human *OAS1* gene and arranged in tandem in the following order: *Oas1e, Oas1c, Oas1b, Oas1f, Oas1h, Oas1g, Oas1a* and *Oas1d*. For all of these eight genes, a specific interferon inducible promoter regulates transcription. So far
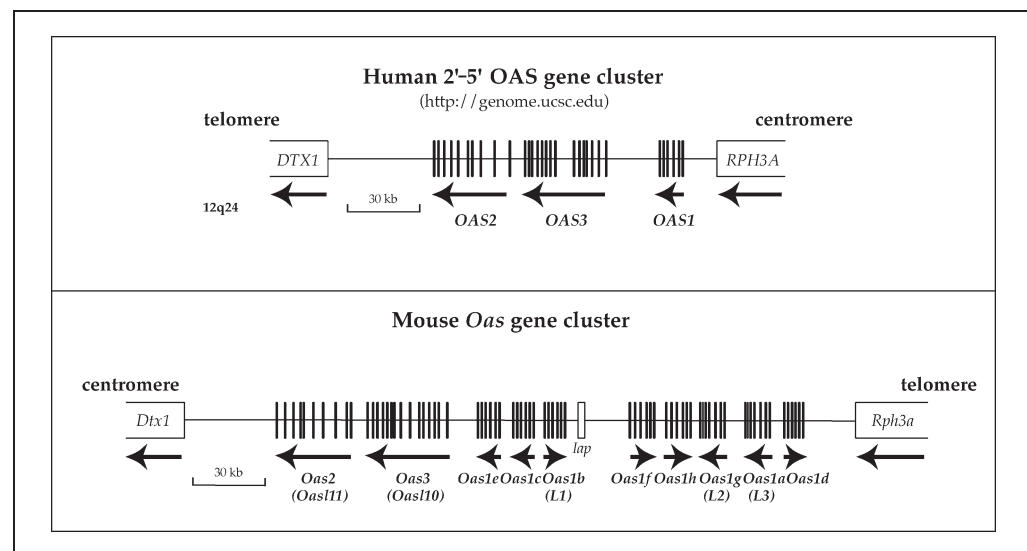
Figure 1: Schematic representation of the organisation of the human and murine clusters of genes encoding 2′-5′ oligoadenylate synthetase (OAS). The picture is not drawn to scale but is correctly orientated with respect to the centromeres for each species. All of these genes are transcribed and the direction of transcription is represented by arrows. Most of the mouse genes are probably pseudogenes, encoding proteins whose function is unknown but are probably not involved in the innate defence mechanisms against viral infections. (From Mashimo *et al.*[10] and reprinted with permission from Elsevier.)

LXXXPA motif is a domain of 9 amino acids at the C-terminus of the protein which is essential for enzymatic activity. CFK motif is essential for tetramerisation of the molecule.

NFκB, GAS and ISRE are acronyms that designate DNA binding sites in the promoter region of the gene.

only *Oas1a* has been found to be alternatively spliced yielding two transcripts each including different parts of exon 6.[19–22]
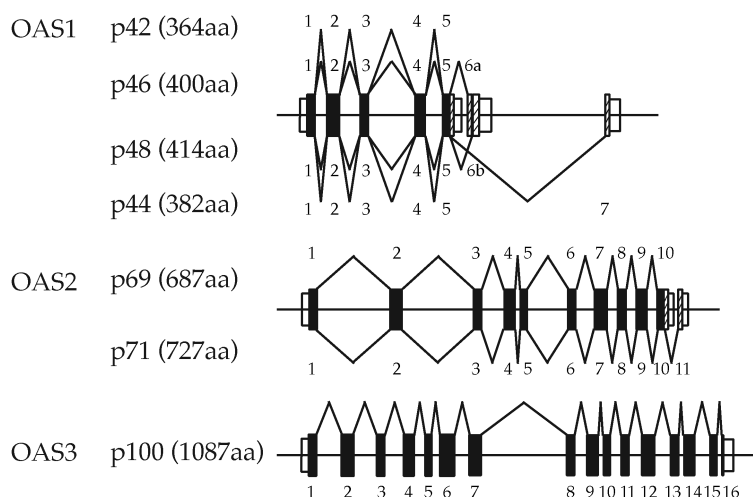
Alignment of the predicted amino acid sequences for the proteins encoded by the eight *Oas1* genes indicates that *Oas1c, Oas1d, Oas1e, Oas1f* and *Oas1h* are structurally very similar and lack functional domains, such as the LXXXPA motif, the highly conserved aspartic acid residues in exon 2 and the CFK motif.[23] These observations suggest that, if these isoforms have retained their binding activity to double-stranded RNA, they have lost their Mg^{2+}–dependent catalytic activity and, accordingly, may actually be inactive pseudogenes.[23–25] By contrast, *Oas1g* and *Oas1a* encode proteins that could be functional in the 2′-5′ OAS/ RNaseL cascade.

The case of *Oas1b* is probably the most interesting of all, since, according to Perelygin and colleagues, the flavivirus–specific activity of the *Oas1b* isoform on viral replication might be correlated with a four–amino acid deletion in the P-loop motif that is unique to this isoform and which does not appear to exist in either rat or human.[11] This four–amino acid deletion may allow the *Oas1b* protein to recognise and bind a specific conserved RNA structure unique to flavivirus RNAs. Even though this hypothesis is supported by other experiments *in vitro*,[26] an alternative explanation to *Oas1b*–specific activity on flavivirus replication may also be found in its promoter sequence, where several binding sites (nuclear factor–κ B, gamma–interferon activation site [GAS] and interferon–alpha stimulated response element [ISRE]) exhibit a unique organisation. In particular, it is noteworthy that *Oas1b* is the only gene for which the two binding sites, nuclear factor–κB and ISRE, are closely associated in tandem, producing a genomic structure that has previously been reported as being capable of triggering gene expression upon viral induction.[27] Sequencing the promoter regions of the *Oas1b* isoform in remotely related mouse species did not provide evidence that a particular structural change in this promoter might be associated with the phenotype of
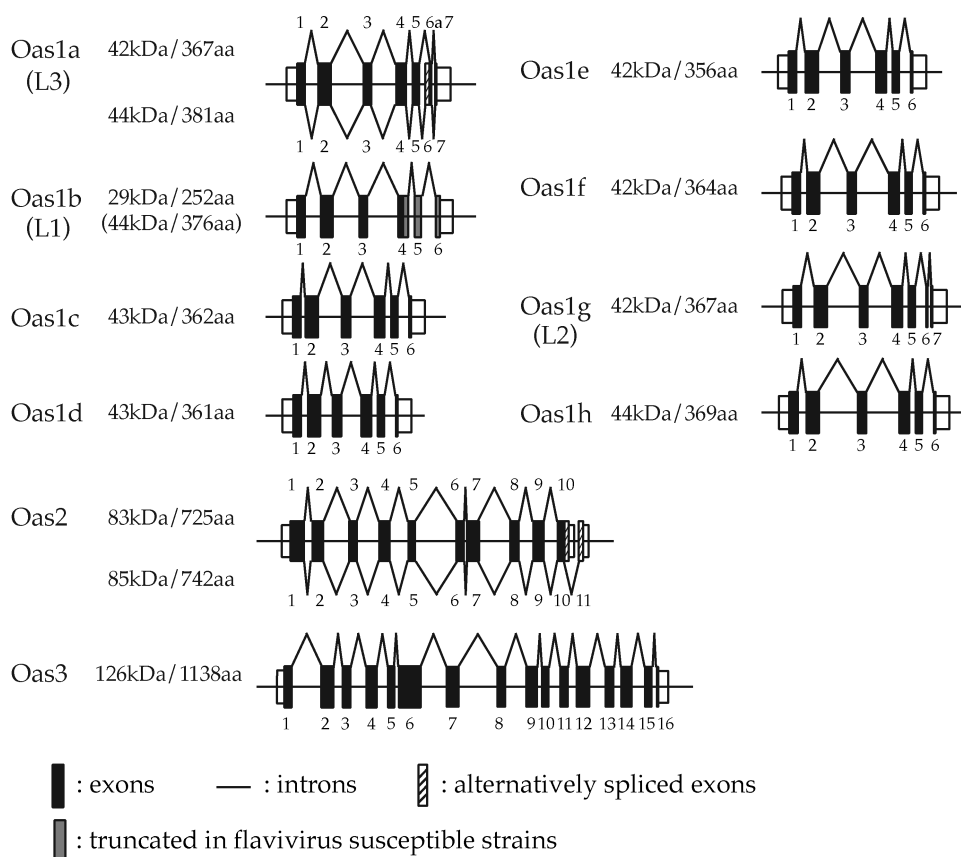
Human OAS transcripts
[Sarker *et al.* 1999, Rebouillat *et al.* 2000,[18] Justesen *et al.* 2000[17]]

OAS1   p42 (364aa)

p46 (400aa)

p48 (414aa)

p44 (382aa)

OAS2   p69 (687aa)

p71 (727aa)

OAS3   p100 (1087aa)

Mouse Oas transcripts
[Mashimo *et al.* 2003[21]]

Oas1a (L3)   42kDa/367aa

44kDa/381aa

Oas1b (L1)   29kDa/252aa (44kDa/376aa)

Oas1c   43kDa/362aa

Oas1d   43kDa/361aa

Oas2   83kDa/725aa

85kDa/742aa

Oas3   126kDa/1138aa

Oas1e   42kDa/356aa

Oas1f   42kDa/364aa

Oas1g (L2)   42kDa/367aa

Oas1h   44kDa/369aa

█ : exons   ── : introns   ▨ : alternatively spliced exons

▌: truncated in flavivirus susceptible strains

Figure 2: Transcription units of the genes encoding 2′-5′ oligoadenylate synthetase (OAS). Scale for exons is five times larger than for introns. Due to a non-sense mutation in exon 4, the *Oas1b/L1* isoform is translated in a truncated form in most mouse laboratory strains, but not in wild mice. Except for *Oas1a*, the mouse specific isoforms of *Oas1* are not alternatively spliced. For isoforms OAS2/Oas2 and OAS3/Oas3, the splicing process is the same in both species. The human 2′-5′ OAS transcripts are referenced according to Justesen *et al.*[17] and Rebouillat *et al.*[18] (From Mashimo *et al.*[10] and reprinted with permission from Elsevier.)

resistance or susceptibility after flavivirus infection. This substantiates the hypothesis that the stop codon found in the *Oas1b* coding sequence of most laboratory strains is directly related to this phenotype.[10]

Situations in which mammalian genomes harbour orthologous genes in variable copy numbers are not uncommon. Olfactory receptors, for example, are also arranged in clusters and are at least three times more numerous in the mouse than in the man.[28] It was suggested that these variations were the result of different selective environmental pressures experienced by the ancestors of modern rodents and primates. Finally, and again concerning the *Oas1b* isoform, a likely hypothesis to account for the presence of the same stop codon in virtually all laboratory strains is that all of these strains inherited the same segment of Chr 5 from a common ancestor. Such a situation is not uncommon among mouse laboratory strains and was also observed by Staeheli and colleagues when, as is discussed below, they elucidated the genetic basis of susceptibility to orthomyxovirus infection.[29] Whether this occurred by chance only or under some sort of selective pressure, however, is an open question.

Infections agents, especially when they are transmitted by arthropods, are not a threat to laboratory animals

Two very interesting observations concerning the genetic control of human susceptibility to flavivirus infections have been published recently. The first, by Bonnevie-Nielsen and colleagues, reports a significant correlation between the basal activity for OAS1 and an A/G SNP at the exon 7 splice-acceptor site (AG or AA) of the *OAS1* gene.[30] According to those authors, in a cohort of 83 families, each containing two parents and two children, allele G had a higher frequency in people with high enzyme activity than in those with low enzyme activity, with the activity being related to this polymorphism in a dose-dependent manner across the GG, GA and AA genotypes. Allele G generates the p46 enzyme isoform, whereas allele A ablates the splice site and generates a dual-

function antiviral/proapoptotic p48 isoform and a novel p52 isoform. The discovery of this genetic polymorphism and of its influence on host susceptibility to flavivirus infections underlines the likely importance of OAS1 in innate mechanisms of defence. Further experiments with mice should now be designed to confirm this interesting hypothesis.

Another epidemiological survey reported by Sakuntabhai and colleagues indicates that polymorphisms at the *CD209* gene, which encodes a C-type lectin differentially expressed by CD8alpha splenic dendritic cells and which is an attachment receptor for the dengue virus, probably also plays a crucial role in the severity of dengue pathogenesis. Dermal/interstitial myeloid dendritic cells constitute the first line of the innate host's defence against pathogens at the anatomical sites where it replicates after the initial bite by the infected mosquito. Consistent with this notion, it has recently been demonstrated that a promoter variant of CD209 (DC-SIGN1-336) has a functional role in the transcriptional regulation of *CD209* and confers strong protection against dengue fever but not against dengue haemorrhagic fever.[31] Engineering mutations in the orthologous mouse gene should also be interesting to test this hypothesis.

MYXOVIRUSES AND BUNYAVIRUSES

The phenotype of innate resistance or susceptibility of mice to flavivirus experimental infections described above was the first example demonstrated to have simple Mendelian inheritance. The phenotype of resistance/susceptibility towards orthomyxoviruses, infections which will now be considered, is the most extensively documented and the first for which the genes responsible have been characterised at the molecular level.[32]

When challenged with mouse-adapted strains of influenza virus, for example when injected intracerebrally with the

neurotropic avian influenza A strain or injected intranasally with a human pneumotropic strain, most laboratory inbred mouse strains die, while mice of strain A2G and most wild-derived inbred strains resist.[33–35] This phenotype is controlled by a genetic region on chromosome 16 with two closely linked genes, *Mx1* and *Mx2*.[36] *Mx1* has three alleles: *Mx1*^{+}, the wild-type allele, which is dominant and confers resistance; and two recessive alleles (*Mx1*^{−}), both resulting in susceptibility. The mouse *Mx1* gene encodes a 72 kDa nuclear protein of the dynamin superfamily of large GTPases, whose transcription is induced by interferon shortly after infection.[29] In *Mx1*^{−}/*Mx1*^{−} mice, the protein is either absent because several exons of the gene are deleted (strain BALB/c, for example), or non-functional because it carries a nonsense mutation (strain CBA, for example).[37] Here again, the mutant, non-functional alleles of *Mx1* are over-represented in laboratory strains but are uncommon in wild mice. It is a good fortune that strain A2G, which was developed from 'illegitimate offspring' of strain A with wild mice, was used in early experiments; otherwise the discovery of the function of MX1, the *Mx1* encoded protein, would probably have been delayed. It is also interesting to note that the phenotype of resistance/susceptibility of wild mice towards viruses of the influenza group was discovered in rather artificial conditions, since mice are not natural hosts for that sort of 'airborne' virus. For this reason, it was suggested that the *Mx1* system may serve an important purpose against other pathogens of the same orthomyxovirus class or against pathogens of related classes. Moreover, it was demonstrated some years later that influenza-like arboviruses, such as the Thogoto virus, the Dhori virus or the Batken virus — which are common pathogens for wild mice — were also able to trigger the *Mx*-mediated innate mechanism of defence.[38–40] Wild mice resist experimental infections with these viruses, whereas laboratory mice do not.

The closely linked *Mx2* gene, which is only a few kilobases apart from the *Mx1* locus, is also non-functional in all laboratory mouse strains examined so far because of an insertional mutation in its coding sequence generating a frame shift and premature termination.[32]

As mentioned above, the MX protein product of the *Mx1* gene is not normally synthesised in resting cells but is induced after interferon-alpha or -beta (but not -gamma) stimulation in macrophages. Unlike 2′-5′ OAS, the MX protein is a nuclear protein whose function is to impede virus replication. Several experiments have clearly demonstrated that the lack of MX1 protein in laboratory mice could be restored by transgenesis with a normal copy of the wild-type *Mx1* allele or the orthologous copy of another species — rat or man, for example.[41–43]

The *Mx2* gene of the feral strains encodes a protein comprising 656 amino acids, which is also expressed following interferon treatment and localises to the cytoplasm. It has been demonstrated that this protein inhibits vesicular stomatitis virus replication.

Similar to 2′-5′ OAS, MX proteins exhibit considerable sequence preservation among most mammalian species, including man. The structure of these proteins has been extensively studied and they have been found to consist essentially of two main domains: an N-terminal domain, which is shared with several other GTP-binding proteins, and a C-terminal domain with two extremely important leucine-zipper motifs, which are essential for protein–protein binding.[44,45] Mutations in either of these two domains usually result in loss of activity for the MX protein. Interestingly, it was demonstrated that some amino acid substitutions can only partially affect the activity of the protein, making it unable to prevent the replication of some, but not all, representatives of the orthomyxovirus family.[46,47] Interspecific variations in the antiviral specificity of the MX protein is

It is likely that many genes cooperate to make a mouse resistant to a particular infection, but these genes can be identified only when they have a mutant non-functional allele

often explained by some discrete polymorphisms at the sequence level.

The MX protein encoded by the *MXA* orthologous gene of human cells shows a high degree of sequence similarity with MX2; it also accumulates in the cytoplasm of interferon-treated cells, associating with the endoplasmic reticulum. Unlike MX1, however, it inhibits a limited range of RNA viruses. It was demonstrated, for example, that transgenic mice that permanently express the human MXA protein became resistant to infection with Thogoto virus but remained susceptible to Dhori virus.[48] This difference in specificity was precisely attributed to a polymorphism in the coding sequence. These results indicate that the *Mx1/Mx2* system is a powerful defence mechanism against tick-borne influenza viruses in mice, although MX1 and human MXA GTPases are also active against some other negative-sense single-stranded RNA viruses such as bunyaviruses (Rift Valley virus, La Crosse virus, Crimea Congo virus, etc).[49] In the case of bunyaviruses, confocal microscopy was used to demonstrate that MXA co-localises with the nucleocapsid protein of the viruses in the perinuclear regions of infected cells, preventing the transport of this protein to the Golgi compartment, the site of virus assembly. In the case of Thogoto virus, meanwhile, MXA prevents the incoming viral nucleocapsids from being transported into the nucleus, the site of viral transcription and replication.[49]

CORONAVIRUSES

Coronaviruses represent a large family of positive-sense and single-stranded RNA viruses. These viruses infect mostly epithelial cells of a wide range of vertebrates, including mice, rats, pigs, cattle, birds and humans. (The recent outbreak of severe acute respiratory syndrome in South-East Asia is still fresh in our memories). In humans, enteric infections can also occur in young infants and neurological syndromes have also been reported. Coronaviruses represent a major threat for laboratory animal (mice

in particular) breeders, with murine hepatitis virus (MHV) being by far the most frequent pathogen in breeding colonies, although the situation has improved over recent years due to better diagnostic and prophylactic measures.

MHV, like other RNA viruses, mutates rapidly and frequently recombines with other coronaviruses of the same group. This results in the generation of a great variety of strains with various degrees of pathogenicity. Among all these strains, the best studied are MHV-1, MHV-2, MHV-3, JHM, A/ 59 and S, of which MHV-3 is regarded as the most aggressive.[50] Some strains have a primary tropism for the upper respiratory tract and others for the enteric mucosa and liver.[51] Mice of all strains and ages are susceptible to experimental infections, but the severity of the clinical symptoms that ensue greatly depends on the genetics of the virus and of the infected strain. Investigations have been undertaken in several laboratories to unravel the genetic determinism of this trait, and two loci have been reported to be of importance: *Hv1* (formerly *Hv*) and *Hv2* (now designated *Ceacam1*). *Hv1* has two alleles: *Hv1^r*, which occurs in strain C3H/An and determines resistance to viruses of the MHV-2 group; and *Hv1^s* which determines susceptibility and is found in strain PRI. Heterozygotes are susceptible. The cellular basis for this phenotype seems to be at the level of macrophages, in which the virus fails to replicate in resistant mice. The molecular basis of this difference is not known and the locus has not even been positioned on the mouse genetic map. It is known, however, that it is neither linked to the *Oas1* locus on chromosome 5 nor to *Hv2*, the other locus for MHV resistance.[52]

Hv2, now designated *Ceacam1*, is more interesting. It was discovered after challenging mice of various inbred strains with viruses of strains JHM or A59.[53,54] Strain SJL carries the resistant allele *Hv2^r* (now known as *Ceacam1^b*), while most other inbred strains are homozygous for the susceptible allele (*Hv2^s*, now

Sequence variations in homologous genes of different species parallel the evolution of infectious agents. It is an illustration of the 'battle' of two genomes.

Ceacam1^a). The *Hv2* locus maps to Chr 7.[55,56]

After a series of elegant experiments, the molecular basis of the phenotype determining susceptibility has now been totally elucidated. The receptor for MHV, designated CEACAM1, is a glycoprotein of the immunoglobulin superfamily and of the carcinoembryonic family of cell adhesion molecules. This molecule is abundant on the intestinal brush border membranes of the colon and small intestine and also on liver cell membranes — two cell types which are the principal targets for MHV replication.[57] This receptor has been isolated by immunoprecipitation and has been extensively studied.[58] It has been demonstrated that it binds specifically to the spikes that are on the envelope (on the corona) of the MHV virus, allowing entry of the virus into the cells. Mice of the inbred SJL strain synthesise an alloform of the glycoprotein (CEACAM1b) which differs from the homologous glycoprotein of the other strains (CEACAM1a) by 27 of the 108 amino acids of the N domain (D1), and this is sufficient to hamper viral integration (and of course replication) into the intestinal and hepatic cells. SJL mice are resistant to 10,000× the normally lethal dose of MHV-A59.[54]

In addition to the murine coronavirus MHV, mouse CEACAM1a protein and its human orthologue are targets of bacterial pathogens such as *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, as well as *Moraxella catarrhalis* in humans. Human coronaviruses, however, do not bind to this molecule.

Mice with a genetically engineered null allele at the *Ceacam1* locus (*Ceacam1$^{-/-}$*) have been produced. It has been demonstrated that these mice are fully resistant to MHV-A59 infections by both intranasal and intracerebral routes:[59] after an experimental infection, virus was recovered from the liver and spinal cord tissues of normal C57BL/6 mice but not of mice homozygous for the knockout

allele. These results indicate that CEACAM1a is the sole receptor for MHV-A59 in both liver and brain, and rules out the possible existence of another receptor for the virus as previously postulated.[60,61] This result is also particularly interesting since it is the first time that a genetically engineered mammalian species has been shown to become resistant to a viral infection and exhibit no other deleterious effects. This, of course, might have considerable economic impact if it could be applied to other species.

HERPESVIRUSES

Herpesviruses represent a large and rather heterogeneous family of enveloped DNA viruses which are a leading cause of diseases in humans, second after influenza viruses. Herpesviruses cause either overt diseases, like varicella or chicken pox, or can remain silent for many years, being reactivated only on certain circumstances, for example as shingles. In the mouse, herpesvirus infections are less common than in humans and apparently only two members of this family can infect spontaneously: MCMV-1, also described as murid herpesvirus 1 (MuHV-1), and the murid herpesvirus 3 or MuHV-3, sometimes described as mouse thymic virus.[50] Only MCMV-1 is interesting in the context of this review because very little is known concerning the pathology of MuHV-3, which seems to be rare and is extremely difficult to grow *in vitro*.

Spontaneous infections of mice by MCMV-1 result in subclinical salivary gland infections in which the virus persists for a very long time, possibly for the duration of the animal's life. In this sense, the infection of mice with MCMV-1 mimics human infection with cytomegalovirus (CMV), which is sexually transmitted and remains almost unapparent, but which can become a serious health problem in patients who are immunodepressed after organ transplantation or if suffering from AIDS.

Experimental infections of mice not previously infected (ie with a specific

Because the infectious agents are sometimes very different, laboratory animals are not always good models for investigating the physiopathology of infectious diseases in humans

patrogen free [SPF] standard) with MCMV-1 can result in the death of the animal or in major symptomatology, depending on the age of the mouse, the strain to which it belongs, the route of inoculation and the dose and strain of virus inoculated.[62]

Variations in susceptibility of the different strains of experimentally infected mice have been reported and a major role was initially attributed to the major histocompatibility complex (MHC) *H2*, with strains with an $H2^b$ or $H2^d$ haplotype at the MHC being ten times less resistant than strains with an $H2^k$ haplotype.[63]

Recombinant inbred strains are inbred strains bred from F1 hybrids between two unrelated parental strains. The genome of these strains is homozygous for 50 per cent of each of the parental alleles.

Scalzo and colleagues, re-investigating the genetic basis for the control of acute splenic replication after inoculation of MCMV virus into mice of strain C57BL/6 (resistant) and BALB/c (susceptible), and using a set of recombinant inbred strains derived from these two parental strains, demonstrated the importance of an autosomal dominant non-*H2* gene, *Cmv1*, with a probable location on Chr 6.[64] The location of the *Cmv1* locus on Chr 6 was confirmed and refined by several authors, and it was demonstrated that the phenotype of resistance/susceptibility was correlated with the activity of natural killer (NK) cells against MCMV-1-infected cells.[65–69] Positional cloning of the *Cmv1* locus revealed that the phenotype was associated with structural variations in a cluster of genes — members of the killer cell lectin-like receptor family a (*Klra*), encoding inhibitory or activating NK cell receptors that interact with MHC class 1 molecules in promoting cytolysis of infected cells.[70] The observation that the recombinant inbred strain BXD-8/Ty, with a $Cmv1^r$ haplotype derived from strain C57BL/6, was nonetheless highly susceptible to MCMV-1 infection, was explained by the discovery of a deletion of the *Klra8* gene, pointing to the specific importance of this gene. Using a panel of monoclonal antibodies, Brown and colleagues demonstrated that the Ly49H was absent in mice of the BXD-8 strain and, moreover, that treatment with an anti-Ly49H monoclonal antibody prior to MCMV infection abrogated MCMV resistance in mice of the C57BL/6 inbred strain.[71] This suspicion was confirmed by producing 'resistant' transgenic mice expressing a functional KLRA8 (formerly Ly49H) in an otherwise susceptible genetic background.[72] The innate defence mechanism operating against MCMV-1 is now well understood: the resistant allele at the *Klra8* locus encodes a membrane receptor capable of binding to a viral product called m157, which is an MHC class I-like protein expressed at the surface of all infected cells, and the m157-KLRA8 interaction triggers the cytolytic machinery of NK cells and the production of interferon-γ.[73,74] Infected mice are then protected by both the killing of infected cells and the production of interferon. The *Klra8* locus provides the first example of an NK receptor that is able to mediate clearance of viral infection via direct recognition of a virally encoded protein.[75] Two interesting observations on this mechanism of defence were published recently. The first, by French and co-workers, indicates that more aggressive mutant viruses can emerge under the selective pressure of innate immunity as it operates in the case of herpesviruses.[76] This, of course, raises a serious issue, since mutant viruses may also occur in human patients and cause death. The second observation originates from Vidal's laboratory at McGill University, from which a great deal of current knowledge about innate immunity to MCMV-1 has been acquired, and indicates that the NK cell mechanism implicated in resistance depends on the functional interaction of the Ly49P receptor with the MHC class I molecule H-2D(k) on MCMV-infected cells.[77] The first observations on the role of *H2* genes have thus been confirmed and elegantly interpreted.

RETROVIRUSES

Retroviruses are special types of RNA virus because, after infection of permissive mammalian cells, they are retrotranscribed as proviruses and integrate into the mammalian genome, where they stay forever. In general, there are three types of retrovirus: the oncoviruses, whose genes are transcribed in oncogenic molecules; the spumaviruses, which are responsible for weak or inapparent infections in many mammalian species; and the lentiviruses, of which HIV1 and HIV2 as the most famous examples, are cytopathogenic. All three classes of retroviruses can infect mice and variations in the susceptibility of the different inbred strains have long been known.[78,79] The Friend leukaemia virus and its many variants have been used extensively as models for studying the intimate mechanisms at work in the determinism of oncovirus susceptibility. From these studies, a number of cellular genes have been identified, either after pure *in vitro* approaches or because they existed in different allelic forms in the different laboratory strains. All of these genes (designated *Fv1, Fv2, Fv4, Rmcf,* etc) actively protect the cells from infection by different mechanisms, acting at different steps of the viral life cycle, and some of them have now been cloned.[80–82] All of these results have been presented in detail in several excellent reviews, so will not be further discussed here.[7,83–86] It is, however, important to understand that research on the susceptibility of mouse cells to adapted retroviruses may have important implications for the development of therapeutic strategies to inhibit other retroviral infections, including those by the lentiviruses HIV1 and HIV2.

OTHER VIRUSES

Several other cases where different strains of laboratory mice exhibit different degrees of susceptibility when experimentally infected have been studied. So far, however, knowledge of these genes has not reached a level of resolution leading to the molecular characterisation of gene products.

Among the most documented cases in which there is a strong influence of host genetics are infections with Theiler's virus. This picornavirus, which is very common in wild mice and is an occasional contaminant of laboratory animal colonies, causes persistent and demyelinating infections of the central nervous system, and this syndrome is considered to be one of the best models of human multiple sclerosis. The virus infects neurones for a few weeks and then shifts to white matter, where it persists in glial cells and macrophages. Susceptibility to persistent infection varies among inbred strains and is multigenic, with a major effect of *H2* class I genes. Other non-*H2* susceptibility loci have been identified, but so far none have been cloned.[87,88] *Tmevp1* and *Tmevp3* are two of these non-*H2* genes that appear to regulate the expression of important cytokines.[89]

Another example is provided by the Sindbis virus, an alphavirus. Based on the observation that BALB/c mice are resistant while C57BL/6 are susceptible, a gene (neuro-adapted Sindbis virus 1 [*Nsv1*]) which controls early viral load and determines the likelihood of paralysis and death has been discovered and mapped to Chr 2, but here again the molecular nature of the protein encoded by this gene is not yet known.[90]

Finally, a few other genes or genetic regions have been found to be of importance for the genetic control of susceptibility of mice to experimental infections with human herpes simplex virus 1. These genes have not even been precisely mapped.[91]

CONCLUSIONS

This review has reported a small number of cases in which different strains of laboratory mice exhibit varying behaviour, with some of them being more resistant than others after experimental infection with several types of viruses. Using the classical strategy of

forward genetics, often called positional cloning, a handful of loci have been identified at the molecular level and the gene products characterised. This has helped to explain why, for example, if the alloform of the CEACAM1 glycoprotein that appears at the surface of intestinal and liver cells of mice does not 'fit' perfectly with the viral ligand, entry of the coronavirus MHV into the cell is impeded and the mouse is not infected. This discovery had an immediate application, with the production of a resistant (and viable) transgenic mouse strain with no glycoprotein at all on its cell surface. Similar situations with other viruses and other species might be discovered in the future. Unfortunately, situations as simple and straightforward as that reported above will probably not be frequent. Viruses are highly host specific, and information gathered from experiments performed in mice with mouse viruses can only be considered as indications or 'targets' for investigations in other species. Human coronaviruses, for example, do not, apparently, bind to CEACAM1-like molecules. Another difficulty is that viruses often use functionally important molecules as receptors, which cannot be deleted or even altered without important side-effects for the host cells. Modifying the cell receptor structure with the aim of altering the phenotype of susceptibility of a given species to viral infection will probably not reveal an easy way to go.

The case of the *OAS1* locus and its importance in the innate mechanism of defence against flavivirus infection is interesting for two reasons. First, the role of $2'$-$5'$ OAS was discovered from observations made in mice, in which *Oas1* exists in two versions: normal, or mutant and non-functional. This finding allowed epidemiologists to focus their attention on this gene, which led to the discovery that the region was critical for the severity of dengue in endemic regions, although a totally different alteration of the molecular structure of the gene was discovered to explain this.[30] It is

likely that situations of this kind will also be found for other viruses. In fact, the genes reported here as examples (*Flv*, *Mx1*, *Mx2*, *Cmv1*, etc) were all discovered because they had a null allele, with dramatic phenotypic effects, segregating the different laboratory strains or wild specimens, but these are exceptions. It is likely that the genes that are involved in the organisation and function of the innate defence mechanisms are extremely numerous, with each of them having an additive effect. Moreover, for the vast majority of these genes, there is, as yet, no mutant, nor even a variant allele amenable to genetic analysis. Many of the transgenic strains that are generated worldwide appear to have an increased susceptibility to the viral infections sometimes occurring in laboratory facilities, irrespective of the nature of the transgene. This clearly indicates a high level of complexity and genetic integration.

A final important point must be addressed in this review, concerning the polymorphism segregation among the laboratory mouse populations. Unlike human populations, laboratory mice are rather homogeneous because they are all derived from a limited number of ancestors stemming from different sub-species.[92] With an increasing use of strains recently derived from wild specimens of unrelated origin, it is likely that many more genes influencing susceptibility towards infectious agents will be discovered. Wild mice are constantly attacked by pathogens, including viruses, and, accordingly, they must constantly improve the specificity and efficiency of their innate mechanisms of defence. Furthermore, these mechanisms probably vary according to the geographical origin of the specimens. In the same way, the chemical mutagenesis or systematic gene-trapping projects that are now in progress in several laboratories worldwide should also provide researchers with many interesting new mutant alleles. The problem in these cases would be to detect the interesting genotypes after challenging

the offspring of mutagenised ancestors with appropriate tests. This will be the challenge for researchers.

Author's Note

While this manuscript was being processed for publication, a paper was released indicating that polymorphisms in the human OAS gene, leading to production of a dominant-negative OASL isozyme similar to the mutant form of *Oas1b* in mice, were associated with increased susceptibility to West Nile infections. *See* Yakub, I., Lillibridge, K. M., Moran, A. *et al.* (2005), 'Single Nucleotide Polymorphisms in Genes for 2′- 5′-Oligoadenylate Synthetase and RNase L in Patients Hospitalized with West Nile Virus Infection' *J. Infect. Dis.*, Vol. 192, pp. 1741–1748.

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