

ECOGENETICS '98

Comparative Genetics of Resistance to Viruses

David G. Brownstein

Section of Comparative Medicine, Yale University School of Medicine, New Haven

Human beings differ widely in their responses to virus infections. Viruses may induce barely discernible symptoms in some patients and severe, life-threatening illness in others. These differences reflect both the complex, intimate, and multifaceted nature of virus-host interactions and the variables that can influence these interactions. Viruses elicit a complex temporal sequence of stringently regulated innate and adaptive immune responses. These responses are essential for recovery, but they also contribute to disease symptoms. Optimal innate and adaptive immune responses are therefore balanced at a narrow interface between ineffectual and overly aggressive. Variables of age, gender, health and immune status, and genetic background influence the availability and form of the host metabolites on which viruses depend; these variables also influence the magnitude and effectiveness of the ensuing host response.

It is well recognized that null mutations of human genes that are essential for innate or adaptive immune responses render homozygotes susceptible to diseases caused by a wide spectrum of viruses. Little is known, however, about the role that more subtle and ubiquitous human gene polymorphisms play in virus-disease resistance. Recent highly publicized reports that individuals who are homozygous for mutant alleles of the CC-chemokine receptor 5 (CCR-5) are HIV-1 resistant has stimulated interest in this area of human genetics (Dean et al. 1996; Samson et al. 1996). CCR-5 is a coreceptor for macrophage-tropic HIV-1. The mutant allele that renders homozygotes refractory to HIV-1 infection carries a 32-bp deletion in an extracellular domain and thereby generates a nonfunctional receptor. This allele is present in ~11% of Caucasian populations; ~1% of the population is therefore homozygous and highly (but not absolutely) resistant to HIV-1 infection. Many other

genetic variations that affect viral receptors are likely to influence susceptibility to other viruses.

Virus-Resistance Genes—Mice and Humans

More is known about genetic control of resistance to viruses in mice than in humans. This is because of both the availability of numerous inbred mouse strains, which vary in their susceptibility to viruses, and the highly developed linkage map of the mouse genome.

Mx1 of mice was both the first virus-resistance gene to be cloned and the first to be used to identify a human homologue. It encodes a type I interferon-inducible nuclear protein that affords specific protection against influenza viruses. Only three mouse strains, A2G, SL/NiA and T9, express the antiviral form of protein; other mouse strains have deletions in the *Mx1* gene that result in truncated proteins that are devoid of antiviral activity (Staeheli et al. 1988). *MxA* is the human homologue of *Mx1* (Staeheli et al. 1985). Like *Mx1*, *MxA* is induced by type I interferon and inhibits influenza-virus replication. Virus specificity for *MxA* is somewhat broader than virus specificity for *Mx1* in that it encompasses vesicular stomatitis virus and measles virus; and, unlike *Mx1*, *MxA* is a cytoplasmic protein (Pavlovic et al. 1995). Although the mechanisms of virus inhibition by Mx proteins have not been determined, they appear to involve GTP binding, GTPase activity, and leucine zippers. Variants of human Mx proteins have not been described, but, given the dramatic impact of the *Mx1* polymorphism on influenza-virus resistance in mice, functional polymorphisms of human Mx proteins could markedly influence resistance to several important viruses.

A Lesson about Genes That Code for Virus Receptors

Hv2, another mouse virus-resistance gene, controls resistance to mouse hepatitis virus (MHV), a large-enveloped RNA virus that is distantly related to human enteric and respiratory coronaviruses. As natural pathogens of mice, MHV strains may cause either localized intestinal infections or generalized infections that emanate from the respiratory tract. All MHV strains appear to use members of the biliary glycoprotein subfamily as

Received December 5, 1997; accepted for publication December 11, 1997; electronically published February 6, 1998.

Address for correspondence and reprints: Dr. David G. Brownstein, Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06510. E-mail: david.brownstein@yale.edu

This article represents the opinion of the author and has not been peer reviewed.

© 1998 by The American Society of Human Genetics. All rights reserved.
0002-9297/98/6202-0002\$02.00

receptor proteins (Dveksler et al. 1993). The principal receptors for MHV are several isoforms encoded by the biliary glycoprotein 1 (*Bgp1*) gene on the proximal end of mouse chromosome 7 (Rao et al. 1997).

The SJL inbred mouse strain, unlike almost all other inbred strains, is highly refractory to MHV infection. The *Bgp1^b* (*Hv2^r*) allele of SJL mice contains divergent sequences in the N-terminal virus-binding domain (Kubo et al. 1994), particularly in the critical residues 38–43 (Rao et al. 1997). The altered *Bgp1^b* isoform remains a functional virus receptor, but its affinity for MHV is reduced by one to two orders of magnitude (Ohtsuka et al. 1996).

Allelic differences of this sort, which reduce but do not abolish receptor affinity, may be much more common than the null-receptor phenotype seen in the *Ccr5-Δ32* homozygotes, especially where the virus receptor has other essential functions. Quantitative binding studies, to compare alleles of genes that encode known viral receptors, may be necessary if alleles are shown to code for functional receptors. Comparatively small differences in the affinities of receptors encoded by different alleles can have dramatic consequences on the course of virus infections in individuals. In the case of the *Bgp1* gene, the 10–100-fold difference in the affinity of the *Bgp1^a* and *Bgp1^b* gene products for MHV translates, after nine replication cycles, into a 10⁹-fold difference in virus titers in the major target organs.

Natural Killer–Cell Receptors

Two virus-resistance genes in mice have been mapped to the natural killer–gene complex (NKC) on distal chromosome 6. The NKC consists of genes that code for receptors that are expressed almost exclusively by natural killer (NK) cells. NK cells are bone marrow–derived large granular lymphocytes, distinct from T and B cells, that serve as antiviral effectors during the early, preadaptive stage of infection. NK cells exert antiviral effects through direct cytolysis of virus-infected cells and production of cytokines.

NK cells lyse virus-infected cells by detecting reduced expression of major histocompatibility complex (MHC) class I molecules. Ligated NK-cell receptors specific for self–MHC class I molecules transduce an inhibitory signal, but, in the absence of the ligand, these receptors fail to repress a pathway that culminates in cytokine release and cytolysis of target cells (fig. 1). In addition to these inhibitory receptors for self–MHC class I molecules, NK cells also express receptors that trigger activation of the cytolytic pathway. Ligands for most activating receptors are not known at present, but they are believed to be ubiquitous host molecules rather than of virus origin.

NK-cell receptors that trigger and inhibit activation are products of two gene complexes that define the

mouse NKC on distal chromosome 6. The NKR–P1 complex encodes activating receptors (Giorda et al. 1990), whereas the Ly49 complex encodes primarily, but not exclusively, inhibitory receptors (Takei et al. 1997). Products of both complexes are type II transmembrane proteins of the C-type lectin superfamily. The NKR–P1 complex consists of at least three genes that are differentially expressed between inbred strains of mice (Giorda and Trucco 1991). The Ly49 gene cluster consists of at least nine genes that encode receptors that share 49%–92% amino acid–sequence homology. At least three Ly49 receptors have specificity for MHC class I molecules and transduce dominant inhibitory signals.

Expression of Ly49 genes in mouse NK cells appears to incorporate a stochastic component that may result in random expression of a set of Ly49 genes in individual NK cells. Each Ly49 receptor is therefore expressed on a subset of NK cells (Höglund et al. 1997). To prevent autoimmunity, each NK cell must express at least one self–MHC class I receptor. In contrast to Ly49, NKR–P1 genes appear to be universally expressed in NK cells, in mouse strains that express them (Giorda et al. 1992).

The two virus-resistance genes, *Cmv1* and *Rmp1*, that have been mapped to the mouse NKC control innate resistance to mouse cytomegalovirus ([CMV] a herpes virus) and ectromelia virus (an orthopoxvirus), respectively. Both groups of viruses have complex DNA genomes that encode host-interactive proteins, many of which are homologues of host proteins. These include homologues of cytokines, cytokine receptors, and, in the case of CMVs, an MHC–class I molecule (Beck and Barrell 1988). This CMV–class I homologue may function to inhibit NK-cell activation by binding to and activating an inhibitory class I–specific receptor.

The *Cmv1* gene has been mapped (Depatie et al. 1997). It has been proposed that *Cmv1*, which maps to the Ly49 gene cluster, encodes alloforms of a Ly49 receptor in resistant and susceptible strains of mice that differ in their affinity for the CMV–class I homologue (Brown et al. 1997). According to this hypothesis, the allele from resistant strains does not bind the viral ligand, so no inhibitory signal is transduced. Alternatively, *Cmv1* or *Rmp1* may encode either inhibitory receptors that are lost in resistant mice or activating receptors that are not expressed in susceptible mice.

The human NKC on chromosome 12p contains several homologues of genes in the murine NKC, but there are significant differences between the two complexes. Human *Nkr-p1* appears to be a single gene that is ~46% homologous with rodent *Nkr-p1*, which codes for an inhibitory rather than an activating receptor (Lanier et al. 1994). Hybridization studies using mouse Ly49 probes have not identified human homologues of Ly49. Two other genes in the human NKC, *Cd94* and *Nkg2*, may affect virus resistance. Both are C-type lectins that

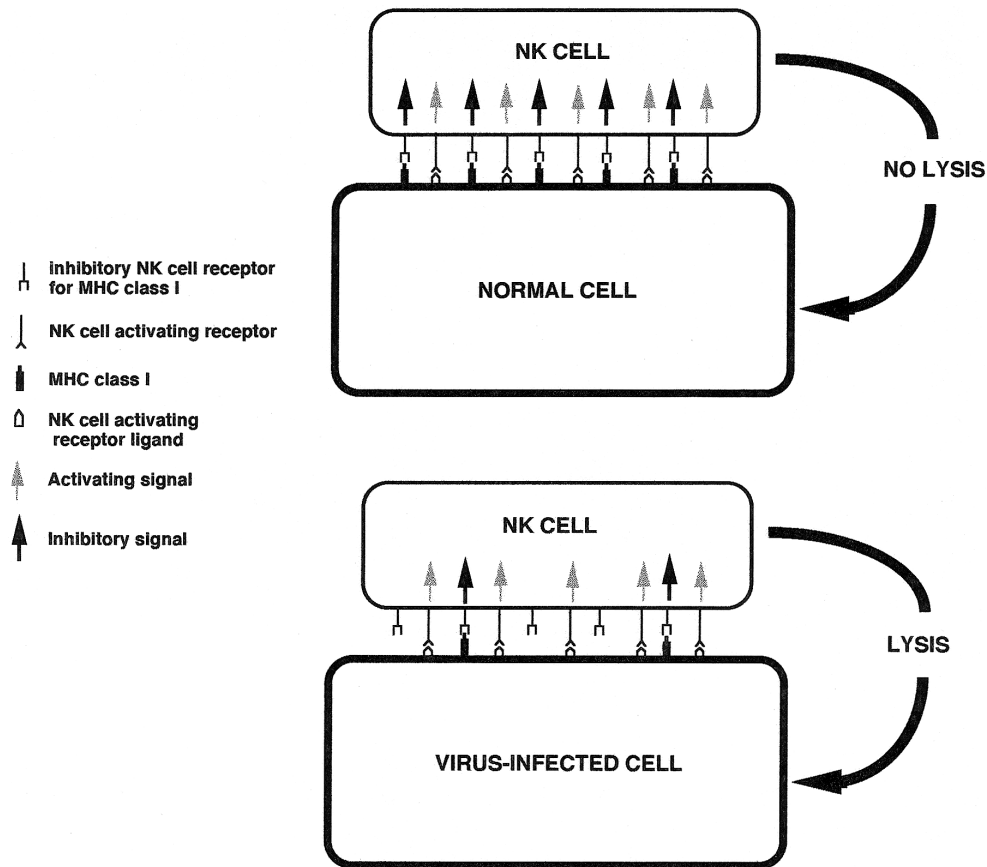


Figure 1 NK-cell recognition of missing self in virus-infected cells. Some NK-cell receptors transduce signals that activate the cytolytic pathway when they bind specific ligands. Other receptors, specific for self-MHC class I molecules, transduce inhibitory signals in normal cells that prevent activation of the cytolytic pathway. When expression of class I molecules is inhibited by replicating virus, class I-specific receptors, in the absence of ligand, fail to repress activation of the cytolytic pathway.

are structurally similar to murine Ly49 and NKR-P1. *Cd94* codes for an invariant molecule that forms heterodimers with the products of the three *Nkg2* genes. At least five cDNAs (A–E) have been identified in the NKG2 family. Depending on which of the NKG2 isoforms is expressed in individual NK cells, the resulting receptor may be activating or inhibitory (López-Botet et al. 1997).

Limits to the Analogy between Mice and Humans

Most HLA-class I inhibitory receptors of human NK cells are not products of the NKC but are expressed by a cluster of genes on chromosome 19q13.4 that code for the killer inhibitory receptors (KIRs). Unlike rodent NKC-encoded MHC-class I inhibitory receptors, human p58, p70, and p140 KIRs are type I transmembrane proteins of the immunoglobulin (Ig) superfamily (Long et al. 1997). Rodent homologues of KIRs have not been identified, perhaps because Ig-like molecules adapted to

class I-receptor function after the divergence of rodent and primate lineages.

Although rodent and human NK cells use different classes of inhibitory class I-specific receptors, downstream signaling events are similar. Both inhibitory rodent Ly49 receptors and human KIRs have cytoplasmic tails that contain immunoreceptor tyrosine-based inhibiting motifs that transduce inhibitory signals by recruiting and activating cytoplasmic protein tyrosine kinases (Burshtyn et al. 1996). Also, KIRs, like Ly49 in rodents, do not undergo somatic rearrangement but are highly complex gene clusters. At least seven genes encode p58 receptors, and p70 receptors are products of a similar number of genes (Moretta et al. 1997). Activating p58-related receptors, named “p50,” are also encoded by genes in this region of chromosome 19. Because of the presence of both activatory receptors and inhibitory receptors with HLA-class I or other specificity, the KIR gene family appears to be a candidate region for virus-resistance genes in humans.

These mouse studies suggest that the human NKC and KIR gene complexes may contain important virus-resistance genes. The specificity of these genes for particular viruses may depend on whether *Cmv1* and *Rmp1* influence resistance by encoding receptors that bind only virus homologues of class I molecules. If receptors encoded by one or both genes bind host ligands, then a wider spectrum of viruses may be influenced by them.

Conclusion

These examples illustrate how mouse-virus systems are being used to identify candidate virus-resistance genes in humans. They provide the probes to detect functional homologues of resistance genes that are shared by rodent and primate lineages. They delineate novel genetic mechanisms of resistance, such as functional but less sticky virus receptors. They also highlight specific genetically complex and polymorphic components of the innate immune system as sources of variation in human responses to viruses. Other benefits are likely to emerge as more mouse virus-resistance genes are identified and characterized. Clearly, not all details of virus-restriction pathways are conserved, but even where differences exist, functional similarities may be enough to guide genetic studies of human resistance to viruses.

References

- Beck S, Barrell BG (1988) Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. *Nature* 331:269–272
- Brown MG, Scalzo AA, Matsumoto K, Yokoyama WM (1997) The natural killer gene complex: a genetic basis for understanding natural killer cell function and innate immunity. *Immunol Rev* 155:53–65
- Burshtyn DN, Scharenberg AM, Wagtmann N, Rajagopalan S, Berrada K, Yi XT, Kinet JP, et al (1996) Recruitment of tyrosine phosphatase HCP by the killer cell inhibitory receptor. *Immunity* 4:77–85
- Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, et al (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CKR5* structural gene. *Science* 273:1856–1862
- Depatie C, Muise E, Lepage P, Gros P, Vidal SM (1997) High-resolution linkage map in the proximity of the host resistance locus *Cmv1*. *Genomics* 39:154–163
- Dveksler GS, Dieffenbach CW, Cardellicchio CB, McCuaig K, Pensiero MN, Jiang GS, Beauchemin N, et al (1993) Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59. *J Virol* 67:1–8
- Giorda R, Rudert WA, Vavassori C, Chambers WH, Hiserodt JC, Trucco M (1990) NKR-P1, a signal transduction molecule on natural killer cells. *Science* 249:1298–1300
- Giorda R, Trucco M (1991) A family of genes selectively coexpressed in adherent lymphokine-activated killer cells. *J Immunol* 147:1701–1708
- Giorda R, Weisberg EP, Ip TK, Trucco M (1992) Genomic structure and strain-specific expression of the natural killer cell receptor NKR-P1. *J Immunol* 149:1957–1963
- Höglund P, Sundbäck J, Olsson-Alheim MY, Johansson M, Salcedo M, Öhlén C, Ljunggren H, et al (1997) Host MHC class I gene control of NK-cell specificity in the mouse. *Immunol Rev* 155:11–28
- Kubo H, Yamada YK, Taguchi F (1994) Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. *J Virol* 68:5403–5410
- Lanier LL, Chang C, Philips JH (1994) Human NKR-P1A: a disulfide linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J Immunol* 153:2417–2428
- Long EO, Burshtyn DN, Clark WP, Peruzzi M, Rajagopalan S, Rojo S, Wagtmann N, et al (1997) Killer cell inhibitory receptors: diversity, specificity, and function. *Immunol Rev* 155:135–144
- López-Botet M, Pérez-Villar JJ, Carretero M, Rodríguez A, Melero I, Bellón T, Llano M, et al (1997) Structure and function of the CD94 C-type lectin receptor complex involved in recognition of HLA class I molecules. *Immunol Rev* 155:165–174
- Moretta A, Biassoni R, Bottino C, Pende D, Vitale M, Poggi A, M.C. Mingari, et al (1997) Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev* 155:105–117
- Ohtsuka N, Yamada YK, Taguchi F (1996) Difference in virus-binding activity of two distinct receptor proteins for mouse hepatitis virus. *J Gen Virol* 77:1683–1692
- Pavlovic J, Arzet HA, Hefti HP, Frese M, Rost D, Ernst B, Kolb E, et al (1995) Enhanced virus resistance of transgenic mice expressing the human MxA protein. *J Virol* 69:4506–4510
- Rao PV, Kumari S, Gallagher TM (1997) Identification of a contiguous 6-residue determinant in the MHV receptor that controls the level of virion binding to cells. *Virology* 229:336–348
- Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, et al (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the *CCR-5* chemokine receptor gene. *Nature* 383:722–725
- Staheli P, Gob R, Meier E, Sutcliffe JG, Haller O (1988) Influenza virus-susceptible mice carry Mx genes with a large deletion or a nonsense mutation. *Mol Cell Biol* 8:4518–4523
- Staheli P, Haller O (1985) Interferon-induced human protein with homology to protein Mx of influenza virus-resistant mice. *Mol Cell Biol* 5:2150–2153
- Takei F, Brennan J, Mager DL (1997f) The Ly-49 family: genes, proteins and recognition of class I MHC. *Immunol Rev* 155:67–77