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Genetic Resistance to Flaviviruses

Margo A. Brinton

I WAS A GRADUATE STUDENT in the Department of Microbiology at the University of Pennsylvania in the late 1960s and chose to do my dissertation research with Hilary Koprowski at The Wistar Institute, even though I was warned that this might not be the best training situation for me. However, because I wanted to work on virus-host interactions and virus-induced disease rather than on the biochemical projects available in other virology labs, I did not change my mind. Dr. Koprowski first assigned me to work on rabies in Tad Wicktor's lab. There I learned the basic techniques of virology and cell culture and worked with both virulent and attenuated strains of rabies viruses in a small cubicle behind a sliding door equipped with a Bunson burner and cotton-plugged glass pipets used for mouth pipetting. Careful technique was very important to becoming a successful researcher. I was later vaccinated with one of the first batches of the new rabies vaccine that was developed at Wistar.

I next began working on my dissertation project, the goal of which was to analyze the characteristics of a flavivirus-specific, genetically controlled resistance phenotype previously discovered in mice. I used West Nile virus as the model flavivirus. Although West Nile virus is now classified as a BSL3 agent, it was considered to be a relatively safe virus when I first worked with it. Koprowski had previously worked with West Nile virus, as well as other arboviruses, in the 1940s at Lederle Labs.[1]

Because of Dr. Koprowski's many other responsibilities, I had to work fairly independently on my research project from the beginning. He traveled frequently and often returned with long lists of suggestions for new directions for my project. Although this input greatly enhanced my education and stimulated my ability to think creatively, I had to quickly learn how to pick out the most relevant and technically feasible of his suggestions. The Wistar environment that Koprowski had created was inclusive, collegial, and international, and many of the scientists at the Institute generously provided me with technical advice and training as needed. It was an incredible privilege for me, as a graduate student, to be able to interact with so many bright and successful scientists. I also had excellent women scientists as role models to emulate, such as Barbara Knowles and Leila Diamond. I also benefited from the excellent seminar series and library at the Institute. In addition, Koprowski had many contacts in the scientific community who provided reagents or collaborations needed for my project.

When I was a doctoral student, an examination in a foreign language was still required by the university. Although I had taken four years of German courses in college, those courses were focused on the humanities, not on scientific literature. I was worried about this exam that typically consisted of reading a scientific paper in German and writing a detailed summary of the contents in English. Dr. Koprowski was fluent in seven languages, including German, and he insisted that he be the faculty member to give me the exam. I spent several months cramming scientific German in preparation for the exam. On the day of the exam, instead of giving me a German scientific paper, Dr. Koprowski handed me a book of German poetry and asked me to orally translate selected poems into English for him. I passed the test, and he enjoyed the exam. Dr. Koprowski felt strongly that to be an innovative investigator, a scientist had to have an additional avocation, preferably in the arts. He encouraged me to continue my training in painting, and, while a graduate student, I attended Saturday classes at the Philadelphia Museum of Art. He would sometimes give me his two Philadelphia Orchestra concert tickets when he could not use them, and these occasions initiated a lifelong love of listening to symphonic music by great orchestras.

The focus of my dissertation project was on a genetically controlled resistance that specifically targets infections by members of the genus *Flavivirus*. This resistance phenotype was first discovered in the 1920s and then rediscovered several times because it was not appreciated that the different viruses being tested all belonged to the same genus.[2–4] One of the early discoverers of this phenotype was Clara Lynch, who smuggled the first Swiss mice into the United States in her purse. When I was writing my dissertation, Dr. Koprowski arranged for me to talk with her to obtain information about the creation of various inbred lines of mice. She was still doing research at the Rockefeller Institute when she was in her 80s. I phoned her at the appointed time, and she answered only a little breathless, having run up several flights of stairs so as not to miss my call. Inheritance patterns in mice indicated that the flavivirus resistance phenotype was conferred by a single autosomal dominant allele named Flv^r. Resistant mice display no symptoms after intracranial inoculation of a flavivirus at doses that kill 100% of susceptible mice. The scope of my dissertation project was broad. It included a survey of the flavivirus susceptibility of inbred mouse strains as well as wild mice, analysis of a linked variation serendipitously discovered while I was working on my project, analyses of the possible involvement of antibody, complement, or interferon in the resistance phenotype, and

Department of Biology, Georgia State University, Atlanta, Georgia.

comparative analyses of viral RNA, viral proteins, and virions produced by resistant and susceptible cells.

Koprowski was intrigued that a host gene variation could have such a dramatic effect on the outcome of infections by a single group of viruses. He quickly realized the advantage of creating congenic resistant and susceptible mouse strains for further study of this resistance phenotype and worked with colleagues to create the resistant line C3H.PRI-Flv^r congenic to susceptible C3H/He mice using a serial backcross protocol with selection for the resistance phenotype at each generation.[5] Although the background variation between the resistant and susceptible congenic mice was greatly reduced, these mice differed by more than a single gene. The length of the donor PRI region containing the *Flv* gene present in the C3H.PRI-Flv^r mice was later estimated to be $\sim$31 centimorgans,[6] and this region was soon shown to contain some additional allelic differences. I had the advantage of using the congenic mice and embryofibroblasts made from them for my dissertation studies.

Although a few flavivirus resistant mouse strains had been generated, the phenotypes of the majority of commonly used inbred mouse strains had not been tested. As part of my dissertation project, I tested ten inbred lines by intracerebral inoculation and found that they were all susceptible.[7] It was also of interest to determine whether the Flv^r allele was rare or widespread in wild mouse populations. Koprowski was able to obtain wild mice trapped in Maryland, Virginia, and California, as well as sera collected from mice at the time of capture, from Robert Huebner. I tested these mice for resistance to flaviviruses and also bred them to susceptible C3H/He mice to determine whether those that were resistant were heterozygotes or homozygotes. None of the sera contained antibody that cross-reacted with WNV. The results indicated that both alleles of the *Flv* locus were segregating in wild mice in the United States.[7] Working with wild mice was very challenging since they jumped directly at you when you opened the cage lid. I spent a lot of time chasing mice around the floor of the animal room until I finally found that a broom containing a small amount of ether lowered gently on a cornered mouse worked well for catching them.

A malfunction of the heating system in the institute's animal facility one winter's night in 1968 caused the ambient temperature to increase to the extent that many of the mice housed in the facility died. Only 8% of the C3H.PRI-Flv^r mice but 58% of the C3H/He mice housed in the same room died. Interestingly, a non-congenic pair of resistant and susceptible strains, BRVR and BSVS, housed in a different room showed similar differential survival. Koprowski wrote a description of this entitled, ''When a thing's really good, it cannot die.''[8] The association between the flavivirus resistance phenotype and resistance to increased ambient temperature in two independently derived mouse strains suggested a possible linkage between these two traits. Koprowski insisted that experiments to confirm this linkage become another component of my dissertation project. Although not a favorite part of my dissertation work, I succeeded in devising controlled experiments using a humidified incubator, and the results confirmed the linkage. Koprowski then recruited Kari Lagerspetz, a Finish scientist who was an expert on temperature regulation and whose son was an accomplished musician, as a collaborator to do follow-up physiological experiments.[7,9] The resistant mice were found to be more efficient at thermoregulation than the susceptible mice but the gene responsible has not yet been identified.

Initially, I used second passage primary embryofibroblasts from resistant and susceptible mice for my cell culture studies but subsequently obtained SV40 from Victorio Defendi's laboratory and successfully generated transformed cell lines. The overall conclusion from a large number of virology and immunology experiments that were done with a variety of techniques was that flavivirus entry into resistant cells is not blocked but virus replication is much less efficient in the resistant cells.[10]

My dissertation work at The Wistar Institute with Hilary Koprowski as my mentor was a wonderful introduction into the exciting world of scientific discovery and excellent preparation for my future career. I learned not to be afraid of taking on new challenges and to follow where my projects led, balancing speculation with practicality. I also learned the benefit of forums that fostered broad scientific communication, which led to my active involvement in initiating new science meetings.

The defense of my dissertation was delayed for 6 months due to Koprowski's sabbatical with Konrad Lorentz, with whom he was studying animal behavior. I began my postdoctoral fellowship and returned to Wistar to defend once he was back. Following my postdoctoral fellowship and a brief period of working on infectious disease drug testing at Riker Labs, Koprowski offered me a staff position with my own lab at the Institute. I will always be grateful for his support in facilitating the initiation of my career as an independent scientist. I obtained funding from NIH to continue working on the flavivirus genetic resistance project and still work on this project today.

Dr. Koprowski shared the C3H/RV mice with investigators at other institutions, and fortuitously the C3H.PRI-Flv^r mice were also found to be resistant to lethal infections with the Gilliam strain of the organism then classified as *Rickettsiae tsutsugamushi* while C3H/He mice were susceptible.[11] Resistance to this organism was previously shown to be controlled by a single, autosomal, dominant allele of the *Ric* locus, which had been mapped using three sets of recombinant inbred mouse strains (BXD, BXH, and BXJ) to chromosome 5 and shown to be closely linked to the retinal degeneration (*rd*) locus.[12] The observed co-segregation of the resistance alleles of the *Flv* and *Ric* loci led to additional recombination studies to fine map the *Flv* locus on chromosome 5 and the identification of a microsatellite that showed no recombination with *Flv*.[13–15] My lab used a positional cloning approach to identify the *Flv* gene. We first identified 22 genes present in a $>$700 Kb DNA contig (the mouse genome sequence was not yet available). After sequencing the coding regions of each gene in congenic resistant C3H.PRI-*Flv^r* and susceptible C3H/He mice, the 2′-5′-oligoadenylate synthetase 1b (Oas1b) gene was identified as the *Flv* locus.[16] The *Flv^r (Oas1b^r)* allele expresses a full length protein (Oas1b), while the *Flv^s (Oas1b^s)* allele expresses a truncated Oas1b (Oas1btr) protein. Most mammalian species have one (e.g., humans) or two copies of the *OAS1* gene but mice and rats have eight adjacent orthologs (Oas1a-h) created by gene duplication.[17,18] Koprowski agreed to serve as editor of our PNAS paper describing these results. Although the mouse Oas1a and Oas1g proteins are functional 2′-5′-oligoadenylate synthetases, the Oas1b proteins are not.[18,19] To confirm that

the truncated product of the Oas1b^r allele was required for the resistance phenotype, we replaced the susceptible Oas1b allele with the resistance allele in a susceptible mouse strain by homologous recombination. The knock-in (KI) C57BL/6 mouse strain (Oas1b-KI-C57BL/6) generated had the flavivirus resistance phenotype, confirming that resistance is conferred by a single Oas1b allele. We recently identified two binding partners of Oas1b,[20] and are continuing to investigate the mechanism by which this very effective natural resistance to flaviviruses functions.

Hilary Koprowski's energy, talents, interests, and achievements always seemed boundless. He was a unique person, and I am very glad I had the privilege of knowing him for more than 40 years. He instilled in me the excitement of science discovery, which I have tried to foster in the students training in my lab. Hilary continued to travel into his 90s and periodically came to Atlanta to attend meetings and give talks at the Centers of Disease Control and Prevention. My husband and I usually took him to dinner on his first night in town. The dinner conversations were always lively, interesting, and covered a broad range of topics, including stories of his latest exploits. Time spent with Hilary was never dull.

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Author Disclosure Statement

The author has no financial interests to disclose.

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Address correspondence to:
Margo A. Brinton
Department of Biology
Georgia State University
PO Box 4010
Atlanta, GA 30302

E-mail: mbrinton@gsu.edu