Double-Edged Genetic Swords and Immunity: Lesson from CCR5 and Beyond

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(See the article by Lim et al, on pages 178–85.)

This other Eden, demi-paradise,
This fortress built by Nature for herself
Against infection
  —William Shakespeare, *Richard II.* Act II, Scene 1

Was Shakespeare, that keen observer of human behavior, also an insightful infectious disease epidemiologist? Had he been prompted to pen these thoughts because he had astutely observed that some individuals resisted acquiring some infections or did poorly once infected compared with others? Validating Shakespeare's crystal ball, 250 years later, it was first postulated that these fortresses are genetic traits, with J. B. S. Haldane and A. C. Allison suggesting that malaria was an evolutionary force that selected for malaria-resistant genes [1]. However, there is a tradeoff. As illustrated by the results reported by Murphy and colleagues in this issue of the *Jour-*

nal [2] and the vignettes described below, it has become apparent that these genetic variants are oftentimes akin to a double-edged sword, serving as a fortress against one infection while conferring susceptibility to another [3] (Figure 1). For example, genetic traits that result in hemoglobin and/or red blood cell disorders (eg, sickle cell disease and thalessemia) protect against malaria. The African-specific allele that results in the null state for Duffy antigen receptor for chemokines (DARC) on erythrocytes protects against *Plasmodium vivax* malaria. However, the role of DARC null state in infectious diseases is likely to be much more complex, because it may correlate with a blunted inflammatory response to endotoxins [4, 5], serve as a genetic basis for the ethnic leukopenia that is observed commonly in persons of African ancestry [6], increase the risk of acquiring human immunodeficiency virus (HIV) infection [7], and confer a survival advantage to leukopenic HIV-positive African Americans [8].

Murphy and colleagues now highlight another genetic tradeoff [2]: the null state of CC chemokine receptor 5 (CCR5) is associated with early symptom development and more pronounced clinical manifestations after infection with West Nile virus (WNV) , whereas this same genetic state is known to confer strong protection against risk of acquiring HIV infection [9].

The CCR5 null state, which is due to homozygosity for the European-specific 32 base pair (bp) coding deletion mutation (Δ32), propelled the HIV field forward in the mid-1990s [9], spawned an explosion of studies that explored the association of *CCR5*Δ32/Δ32 with a myriad of infectious and noninfectious diseases, and led to the development of CCR5 blockers for the treatment of HIV disease.

In retrospect, defining the link between CCR5 surface expression and HIV pathogenesis appears to be Act I of a 3-act Shakespearean play on the role of CCR5. Act II is punctuated by scenes that reveal the double-edged nature of the phenotypes associated with the possession of the *CCR5*Δ32/Δ32 genotype. From a historical perspective, it is noteworthy that the Murphy laboratory has played a major role in both acts thus far. In Act I, his laboratory was among the first to clone and functionally characterize CCR5 [10] and, along with the Berger laboratory, demonstrate that CCR5 is the major coreceptor required for cell entry of HIV-1 [11]. In Act II, although CCR5-null mice were found to have immune perturbations following inflammatory challenges [12, 13], the plot really heated up when the Murphy laboratory challenged these mice with WNV [14], a mosquito-borne neurotropic flavivirus. From this point onward, the story resembles *Macbeth*.

Figure 1. The yin-yang of infectious disease alleles. The concept of yin and yang illustrates the detrimental (*black region*) and beneficial (*white region*) effects of different infectious disease–influencing alleles in the human genome. Illustrated are the *CCR5Δ*32 allele, Duffy antigen receptor for chemokines (DARC)–null allele (*FYO*), and the sickle cell allele (*HbS*). HIV, human immunodeficiency virus; WNV, West Nile virus.

Murphy's group showed that after challenge with WNV, CCR5-null mice had markedly increased viral titers in the central nervous system and had increased mortality [14] compared with that of wild-type mice, thus suggesting that CCR5 expression was necessary to mount a strong host defense against WNV. Subsequently, they demonstrated that there was a strong epidemiologic association between homozygosity for *CCR5Δ*32 and WNV in humans [15, 16].

However, because they were unable to distinguish in their previous studies whether the observations were associated with susceptibility to acquiring WNV or associated with the severity of clinical presentation, they conducted the present study [2]. Lim et al [2] now show that the prevalence of *CCR5Δ*32/Δ32 was comparable in case patients with WNV infection and control participants, which suggests that this genotype is not a susceptibility factor for acquiring WNV infection. However, among the case patients, those patients who were homozygous for *CCR5Δ*32 experienced significantly more symptoms, on average, than did those patients who were heterozygous for *CCR5Δ*32 or who had wild-type *CCR5* genotype. These data indicate that the CCR5 null state is a risk factor for more pronounced early clinical manifestations after infection with WNV [2].

A noteworthy aspect of the present report by Lim et al [2] is the study design. The case patients and control participants were derived from ~35 million blood donors who were screened for WNV [2]. This contrasts with their prior studies, which examined subjects who sought medical attention for symptomatic disease and were compared with otherwise healthy subjects [15, 16]. Also minimizing selection bias, both case patients and control participants in this report were administered the same standardized symptom questionnaire before disclosure of their true WNV infection status [2], and this study feature facilitated evaluation of the association of the CCR5 null state with the number and severity of early symptoms of WNV infection.

What will Act III reveal? Readers are referred to some possibilities posited in recent opinion pieces [17–19]. We focus on 4 points. First, the present study raises a pathogenic conundrum: why does the CCR5 null state confer risk for a more aggressive disease but not associate with risk of acquiring WNV infection (ie, viral entry)? One possibility is that CCR5-mediated signaling events generate critical immune responses that contain the spread of infection but are irrelevant for the initial entry of WNV. In this regard, there are abundant in vitro data linking CCR5 and its ligands to T cell immunity, and 2 recent studies provide corroborative in vivo data: first, that both humans and mice lacking CCR5 surface expression display reduced delayed-type hypersensitivity skin test responses (an in vivo correlate of T cell function and interleukin 2 [IL-2] production [20, 21]), and second, that CCR5 expression regulates T cell proliferation, as well as IL-2 and CD25 expression during T lymphocyte activation [22]. Notably, T cells from CCR5-null mice secrete lower amounts of IL-2 than do wild-type mice; a similar phenotype is observed in

CCR5Δ32 homozygotes, as well as after Ab-mediated blockade of CCR5 in human T cells genetically intact for CCR5 expression [22]. These studies underscore that CCR5 expression may influence clinical outcomes after viral infection by affecting parameters (eg, T cell immunity) that are independent of viral entry [21, 23]. This may have relevance to antiviral immune responses to flaviviruses, including WNV, because CD4⁺ T cells have a critical function in the control and resolution of primary WNV infection; a strong Th1 T cell response, as characterized by interferon and IL-2 production, results in reduction of neurological sequelae [18, 24, 25]. Thus, one possibility is that the phenotype of "low CCR5 expression–low IL-2 levels" may contribute to WNV pathogenesis. Hence, we anticipate that Act III will define the precise mechanisms by which CCR5 influences antiviral responses to flaviviruses as well as to lentiviruses.

Second, the possible consequences of infection with WNV or other flaviviruses in HIV-positive patients who are receiving CCR5 blockers remains unknown, because very little is understood regarding the long-term effects of CCR5 blockers on immune functions in vivo. A previous study found that Maraviroc, a CCR5 antagonist, did not influence IL-2 and CD25 levels, whereas germ-line inactivation of CCR5 and Ab-mediated blockade of CCR5 did influence IL-2 and CD25 levels [22]. This may have been due to differences in the receptor configuration and resulting functionality of Ab-bound and inhibitor-bound forms of CCR5 [26, 27]. Hence, it is conceivable that the effects on immune function secondary to germ-line absence of CCR5 in humans and mice versus chemical antagonism of CCR5, such as after administration of Maraviroc, are dissimilar. Given that distinct biological responses of CCR5 might be determined through different receptor conformations [28, 29], presumably the signaling pathways triggered in cells exposed to Maraviroc versus cells genetically lacking CCR5 may be distinct. Highlighting this possibility is the recent observation that CCR5 forms hetero-oligomeric complexes with at least 2 other chemokine receptors (CCR2 and CXCR4), and specific antagonists of 1 set of receptors (eg, CCR2 and CCR5) lead to functional cross-inhibition of the other (ie, CXCR4) [30]. This has relevance to the full evaluation of the health consequences of CCR5 blockers, because these data suggest that antagonists of 1 chemokine receptor may regulate the functional properties of another to which they do not directly bind [30]. Thus, Act III may reveal that the immune consequences of CCR5 blockers may not be identical to those found in CCR5-null people.

Third, the studies by the Murphy group pose a dilemma. Is there a threshold of CCR5 expression, albeit low, that promotes WNV disease? At least in the context of HIV infection, there appears to be a threshold of CCR5 surface expression that is permissive for cell entry, such that small changes in CCR5 density are associated with large increases in HIV infectivity and efficacy of CCR5 blockers [31–37]. The converse may be operative in WNV infection, in that CCR5 expression levels below a certain (low) threshold may enhance the risk of a more aggressive WNV clinical presentation. Whether such a threshold of CCR5 expression exists is a testable hypothesis because subjects bearing one or lacking the CCR5Δ32 allele display a wide range of CCR5 surface expression levels [38], and this variability may be partly due to CCR5 promoter polymorphisms that influence expression [39–41]. Additionally, one may also need to consider other factors that result in low CCR5 expression levels. For example, the copy number of CCL3L1, a potent CCR5 agonist, correlates inversely with CCR5 expression [42]. Hence, Act III may clarify this dilemma.

Finally, we anticipate that Act III will continue to be punctuated by additional examples that show the tradeoffs associated with the CCR5 null state. This is already happening: a recent study showed that CCR5Δ32 homozygosity is associated with increased susceptibility to tick-borne encephalitis virus [43]. These tradeoffs are a reminder of the constant tug of war between host and pathogen and also of the need to be vigilant, because what we find in one context might differ in another.

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