

A Coevolutionary Arms Race between Hosts and Viruses Drives Polymorphism and Polygenicity of NK Cell Receptors

Paola Carrillo-Bustamante,^{*1} Can Keşmir,¹ and Rob J. de Boer¹

¹Theoretical Biology & Bioinformatics, Department of Biology, Utrecht University, Utrecht, The Netherlands

***Corresponding author:** E-mail: paolacarrillob@gmail.com.

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Abstract

Natural killer cell receptors (NKR) monitor the expression of major histocompatibility class I (MHC-I) and stress molecules to detect unhealthy tissue, such as infected or tumor cells. The NKR gene family shows a remarkable genetic diversity, containing several genes encoding receptors with activating and inhibiting signaling, and varying in gene content and allelic polymorphism. The expansion of the NKR genes is species-specific, with different species evolving alternative expanded NKR genes, which encode structurally different proteins, yet perform comparable functions. So far, the biological function of this expansion within the NKR cluster has remained poorly understood. To study the evolution of NKRs, we have developed an agent-based model implementing a coevolutionary scenario between hosts and herpes-like viruses that are able to evade the immune response by downregulating the expression of MHC-I on the cell surface. We show that hosts evolve specific inhibitory NKRs, specialized to particular MHC-I alleles in the population. Viruses in our simulations readily evolve proteins mimicking the MHC molecules of their host, even in the absence of MHC-I downregulation. As a result, the NKR locus becomes polygenic and polymorphic, encoding both specific inhibiting and activating receptors to optimally protect the hosts from coevolving viruses.

Key words: NK cell receptors, coevolution, host–pathogen arms race.

Introduction

Natural killer (NK) cells are key players in the immune defense against viral infections and tumors (Vivier et al. 2008). Classified as components of the nonspecific innate immune system, NK cells recognize abnormal or viral infected cells with a sophisticated array of germline-encoded activating and inhibiting receptors. Inhibiting NK cell receptors (iNKRs) bind mostly major histocompatibility class I (MHC-I) molecules, providing a mechanism by which NK cells distinguish normal healthy cells from abnormal cells expressing reduced levels of MHC-I molecules on the cell surface. Activating NK cell receptors (aNKRs) target viral products, stress-induced ligands, or other tumor-specific molecules (Lanier 2005). Thus, the engagement of iNKRs and aNKRs enables NK cells to become cytotoxic toward abnormal or viral infected cells, whereas remaining silent toward healthy cells with normal MHC-I expression.

Some of the NK cell receptors (NKR) discriminating between healthy and unhealthy cells are highly conserved across species. Examples are the inhibitory CD94/NKG2 receptors recognizing the also monomorphic human leukocyte antigen (HLA)-E in humans and Qa-1^b in mice (Borrego et al. 1998; Braud et al. 1998; Petrie et al. 2008; Zeng et al. 2012), and the activating NKG2D detecting MHC-I-like proteins that are induced upon stress, like MIC-A, MIC-B in humans and RAE-1, MULT1 and H60 proteins in mice (Bauer et al. 1999; Cerwenka et al. 2000; Cerwenka and Lanier 2001; Bacon et al. 2004). In addition to these conserved receptor-ligand interactions, there exists a variety of polymorphic multigene

families performing equivalent functions. These include the killer immunoglobulin-like receptors (KIRs) in higher primates (Guethlein et al. 2007; Parham et al. 2010), the CD94/NKG2 receptor family in lemurs (Averdam et al. 2009), and the Ly49 gene family in rodents and equids (Wilhelm et al. 2002; Wilhelm and Mager 2004).

The multigene NKR families exhibit a large variation on the population level, as haplotypes differ in gene content, allelic polymorphism, and the ratio between aNKR and iNKR genes. Because NKR genes segregate independently from MHC-I genes, the number of possible receptor-ligand combinations is enormous. But, if conserved receptor ligand interactions (such as NKG2A/HLA-E, NKG2A/Qa-1^b, and NKG2D/MIC-A) are able to successfully discriminate between self and viral molecules, what is the evolutionary advantage of having polymorphic, polygenic, and specific NKRs? The polymorphism of their ligands (i.e., classical MHC-I molecules) is not enough to explain the polymorphism of NKRs, as there are several nonpolymorphic receptors binding all class I MHC molecules (e.g., CD8 on T cells). Thus, what is the selection pressure driving the evolution of polygenicity and polymorphism within the NKR cluster?

The fact that different species show expansion of different structural NKR families suggests that the NKR cluster has been subject of rapid evolution and that a polygenic and polymorphic NKR gene complex provides an evolutionary advantage in many taxa. Moreover, the associations between specific NKRs and positive disease outcome in human viral infections, such as HIV-1 (Martin et al. 2002; Pelak et al. 2011)

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and HCV (López-Vázquez et al. 2005), further suggest that NKR are beneficial against a large number of viral infections. The complex evolutionary interplay between NKRs and viruses has been elucidated by several studies of mice infected with the murine cytomegalovirus (MCMV) (Arase et al. 2002; Smith et al. 2002). MCMV downregulates the expression of MHC-I and additionally codes for MHC-like molecules that can engage iNKRs in 129/J mice, thereby impeding NK cell activation (Smith et al. 2002). In contrast to 129/J mice, C57BL/6 mice are resistant to MCMV infection. The susceptibility of 129/J mice has been explained by the strong interaction of their iNKR Ly49I with the CMV-encoded protein m157. In contrast, the resistance of C57BL/6 mice to MCMV has been genetically mapped to a gene coding for the aNKR Ly49H, which also binds m157 with high affinity (Lee et al. 2001; Smith et al. 2002). Because the activating Ly49H evolved from its inhibitory counterpart Ly49I (Abi-Rached and Parham 2005), it has been proposed that the evolution of the aNKR resulted from the immune pressure exerted by CMV having escaped the NK cell responses by evolving “MHC-I decoys” (Arase and Lanier 2002; Lanier 2008; Sun and Lanier 2009).

C57BL/6 is not the only strain resistant to MCMV. Inbred MA/My mice also have low viral titers after infection with CMV and do not possess the Ly49H gene. Their resistance is mediated by the aNKR Ly49P which specifically recognizes MCMV infected cells in a H2-D^k dependent manner (Kielczewska et al. 2009). Interestingly, this resistance requires the presence of the virally encoded protein m04, which escorts and binds newly assembled MHC-I molecules on the cell surface. Other aNKRs that recognize CMV infection in a m04-H2-dependent manner include Ly49L^{BALB}, Ly49P1^{NOD}, and Ly49W1^{NOD} (Pyzik et al. 2011). Like m157, the original function of m04 might have been to counteract the effect of MHC downregulation and avoid “missing-self” detection by iNKRs. Apparently, hosts evolved novel aNKRs allowing them to control the infection as a counterstrategy.

The hosts’ adaptation exerts a strong selection pressure on the virus, as demonstrated by the high degree of polymorphism observed in the m157 and m04 genes from MCMV strains isolated from wild mice (Voigt et al. 2003), as well as by the loss-of-function mutations in m157 genes after serial passage of MCMV in C57BL/6 mice (Voigt et al. 2003). Thus, the coevolution between NKRs and viruses shows features of Red Queen dynamics, where viruses continue to evolve immunoevasive mechanisms to escape from the NK cell response, and the hosts keep on adapting by evolving novel NKRs, exerting novel selection pressures on the virus.

We investigate this dynamic and complex coevolutionary process with a computational agent-based model (ABM) of host populations infected with two different viral species, one of which is able to evade the adaptive immune response by downregulating MHC-I expression. We observe that viruses “spontaneously” evolve MHC-like molecules, “fooling” and hence escaping detection by both iNKRs and aNKRs. Consequently, hosts evolve haplotypes composed of specific iNKRs, which are specialized to recognize small groups of

MHC-I molecules, and sufficiently specific aNKRs, recognizing viral molecules.

Results

Agent-Based Model

To study the evolution of NKRs in a host population, we developed an ABM based on our previously described models (Carrillo-Bustamante et al. 2013, 2014). Briefly, the host population consists of simplified humans infected with nonlethal viruses causing chronic infections. The hosts are diploid, carrying two polymorphic MHC loci and an NKR cluster. NKRs and MHC molecules are modeled with random sequences of zeros and ones (i.e., bit strings) as a simplified representation of amino acids. Whenever the longest adjacent complementary match between two strings exceeds a binding threshold, the molecules can interact ([supplementary fig. S1A, Supplementary Material online](#)).

Initially, the NKR cluster is composed of one degenerate receptor pair, that is, one iNKR and one aNKR, each recognizing every MHC molecule in the population. Upon birth, individuals inherit one NKR haplotype from each parent. During this process, NKRs can mutate their sequence, their binding threshold, and their signaling potential, allowing for the emergence of novel receptors. If a newly generated receptor is so specific that it fails to recognize any MHC molecule in the population, it will be a “pseudogene.” We focus on the evolution of NKRs, and therefore fix the MHC polymorphism throughout the simulations. The MHC alleles in the population are not completely random, but somewhat similar to each other (see Materials and Methods).

During development, NK cells undergo an education process during which their reactivity is “tuned.” Although the binding of iNKRs with their cognate MHC molecules renders these NK cells functional capacity (Anfossi et al. 2006; Chalifour et al. 2009; Elliott and Yokoyama 2011), the exposure of aNKRs to self ligands results in hyporesponsive cells (Sun and Lanier 2008; Fauriat et al. 2010). We implement this process at birth by evaluating the binding of NKRs with their MHC ligands in the host. For simplicity, we do not model individual NK cell subsets, but estimate the total repertoire of “licensed” receptors per host. In each host, the repertoire of licensed receptors consists of iNKRs binding to at least one of the host’s MHC molecules, and aNKRs failing to recognize all of the host’s MHC molecules ([supplementary fig. S1B, Supplementary Material online](#)). Additionally, aNKRs can only become licensed if the host expresses at least one licensed iNKR. This last point was included to mirror the fact that the presence of licensed aNKRs only would result in NK cell-related autoimmunity; however, this does not have a large impact on our results. In our model, only licensed NKRs participate during an immune response, assuming that at least one NK cell subset will express the set of licensed receptors, and will become activated and expand upon infection.

We model two viral species causing chronic infections (viral species A and B). Both species carry molecules (also modeled with bit strings), representing products coded by

viral genomes. Both viral species can mutate their viral molecules during the transmission to a new host, allowing for the emergence of new strains of each species. B viruses can escape the cytotoxicity of T cells by downregulating the expression of MHC-I on infected cells. Upon transmission, the host will enter a phase of acute infection, after which it can either recover or become chronically infected. Individuals clearing an infection become immune against that particular viral species for a period of 10 years. Hosts can be coinfecting with one viral strain of each species, but we do not allow for superinfection with different strains of the same virus.

The probability of clearing the infection depends on the viral strategy of the virus to escape the immune response (see Materials and Methods). If the viral molecules can be detected by at least one aNKR of the host that gets infected, the NK cells of that individual will receive an extra activating signal. However, if the viral molecule of the B species engages at least one of the licensed iNKRs, the NK cells of that host will not be able to detect the altered MHC-I expression, and will be inhibited. Thus, we assume different levels of protection depending on the possible combinations of iNKRs and aNKRs per individual (illustrated in [fig. 1](#) and explained in Materials and Methods).

In a typical simulation, 100 different strains from each viral species are introduced after the population has stabilized ($t_1 = 10,000$ years). Very rapidly, the viruses spread through the population, infecting almost every individual, and causing a drastic reduction of the population size ([fig. 2A](#), black line). In the infected subpopulation, most hosts are infected with strains of the B species because it is more challenging to become immune against B viruses compared with A viruses, as B viruses downregulate MHC-I molecules ([fig. 1](#)). After several host generations, a population of individuals that is immune against both viral strains evolves ([fig. 2A](#), green line). The number of immune individuals increases over time, causing a recovery of the total population size, suggesting that the initially susceptible host population evolves an NKR system providing immunity to both types of infections.

Immunity to Infections Is Associated with the Expansion of Specific iNKRs and aNKRs

To understand how NKR evolution enables the immunity of the host population, we analyzed the receptors in the NKR cluster before and after a long period of coevolution ([fig. 2B](#)). The initial haplotype composed of one degenerate aNKR and one degenerate iNKR diversifies shortly after the introduction of the viruses, with a clear selection for large mixed haplotypes. Additionally, we observe selection for polymorphism, with populations evolving more than nine different haplotypes as estimated by the Simpson's Reciprocal Index (SRI) diversity measure (see Materials and Methods).

As more receptors evolve, the number of immune individuals increases, indicating that an expanded haplotype is necessary for the hosts to clear the infections. At the end of the simulations, the fraction of hosts responding well to both types of viruses increases compared with hosts from the initially susceptible population ([fig. 3A–C](#)), confirming that

hosts evolve better immunity. The observation that the number of immune individuals increases as the haplotype composition changes was consistent for 15 simulations ([fig. 3A–C](#)).

To better study the effects of each viral species, we also performed simulations of populations spreading strains of only one viral species ([supplementary fig. S2, Supplementary Material](#) online). Populations infected with A strains only expand their aNKRs while keeping one degenerate iNKR ([supplementary fig. S2B, Supplementary Material](#) online). This is because iNKRs remain engaged by the host's MHC-I molecules as A viruses do not downregulate MHC. On the other hand, there is selection for aNKRs recognizing less MHC-I molecules, as they are advantageous only if they become licensed within an individual by not binding any self MHC-I molecule.

During infections with viruses downregulating MHC-I expression (i.e., B strains), the specificity of iNKRs plays a crucial role. If an iNKR binds the viral molecule, the virus successfully masks MHC downregulation, and escapes the NK cell response. As iNKRs should learn to discriminate between self MHC-I and viral molecules, there is selection for iNKRs binding fewer MHC-I molecules in the presence of B strains ([supplementary fig. S2D, Supplementary Material](#) online). The disadvantage of having specific iNKRs is that they are less likely to bind an MHC-I in the same host, reducing the repertoire of licensed iNKRs in the host. The selection pressure to have licensed iNKRs that can detect MHC downregulation drives the evolution of large haplotypes, as a haplotype encoding several specific iNKRs is more likely to contribute licensed iNKRs.

Thus, our simulations show that in order to survive an infection with different viruses downregulating MHC-I, a single degenerate receptor pair is not sufficient. Immunity evolves by increasing the haplotype composition with specific aNKRs and iNKRs.

iNKRs Specialize to MHC Molecules

The previous analysis shows that NKRs evolve to recognize a small fraction of MHC-I molecules in the population. As the probability of an NKR interacting with its ligands, that is, its specificity, depends on its binding threshold, we first measure the binding threshold of aNKRs and iNKRs before (i.e., $t_1 = 10,000$ years) and after the infections (i.e., $t_{\text{end}} = 3$ My). As expected from the haplotype analysis, the binding threshold of both receptor types increases after the introduction of the viruses ([fig. 4A](#)), confirming the evolution of specificity.

To further analyze the specificity, we next determine the complementarity of NKRs to the MHC-I molecules. To this end, we measure the maximal complementary adjacent match between each evolved NKR and the 30 MHC alleles in the same population (see Materials and Methods). A random NKR is expected to bind maximally 30% of random MHC molecules with a maximal complementary adjacent match of 3 bits, as predicted by the frequency distribution of MHC molecules recognized with a particular complementary adjacent length ([fig. 4B](#), blue curve).

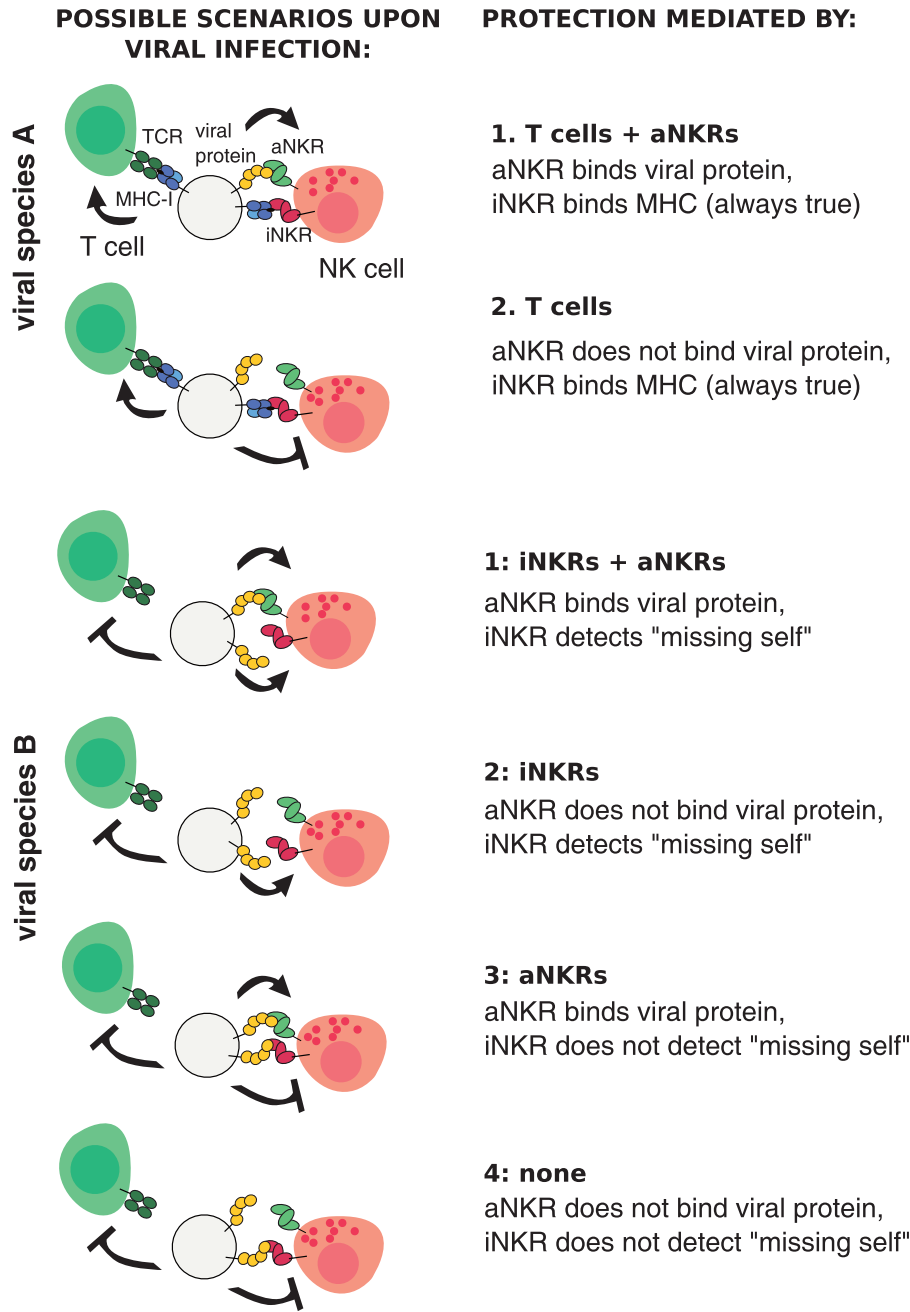


Fig. 1. Model cartoon of NK cell subsets interacting with viral infected cells. Individual NK cells can detect viral infected cells with their iNKRs (depicted in red) and aNKRs (depicted in green). Depending on the interactions between NKRs and the expressed viral molecules (shown in yellow), some subsets of NK cells will be inhibited (bar-headed arrows) and/or activated (arrows). There are two possible NK cell responses against viral species A, and four possible ones against viral species B. Note that we do not explicitly model individual NK cells as depicted here, but we consider a global repertoire of licensed NKRs to determine the levels of protection within a host (see Materials and Methods and table 1).

Similarly, a random NKR should not bind more than 5% of random MHC molecules with a maximal complementary match larger than six. In our simulations, the frequency distribution between the evolved NKRs and their own MHC molecules deviate from this expectation, with iNKRs recognizing more than 2-fold more MHC molecules at their evolved binding threshold (fig. 4B, black curve). Interestingly, the binding of the same evolved iNKRs to randomly generated MHC molecules does not deviate from the expected values (fig. 4C), suggesting that iNKRs evolve to specialize to particular MHC molecules of the population.

On the contrary, the complementarity of aNKRs to their cognate MHC molecules does not deviate from the expectation. These results suggest that there is selection pressure for iNKRs to specialize to particular MHC-I alleles. Indeed, carrying specialized iNKRs is advantageous for the hosts, as it enables iNKRs to detect a diverse array of MHC alleles, while minimizing the interactions with viral molecules.

Viruses Evolve MHC-I Mimics

We next studied the evolution of the viruses. When analyzing the most dominant viruses, we found that they evolve to

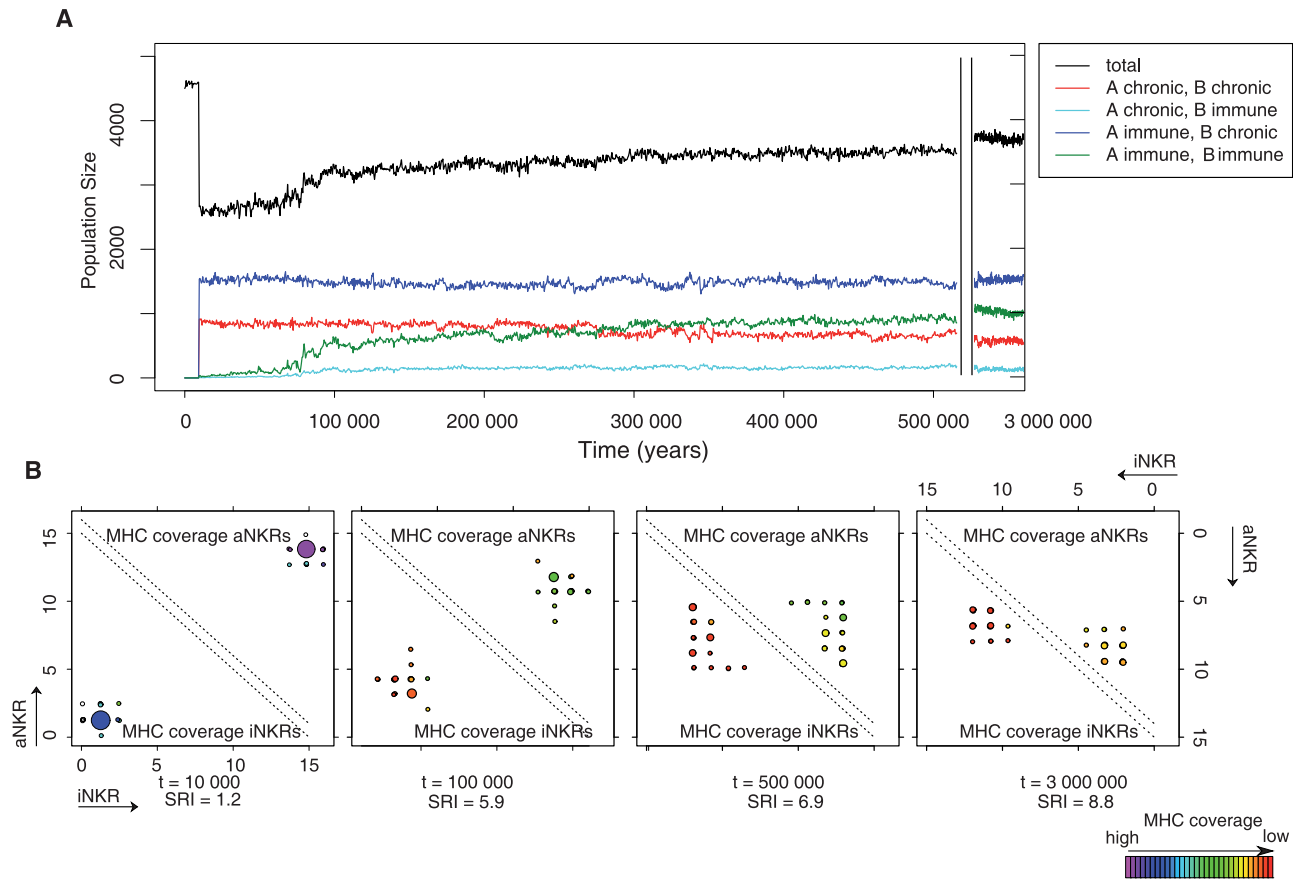


Fig. 2. A host population evolves immunity to two viral species due to an expansion of the NKR cluster. (A) Single representative simulation of a population infected with both viral species. After their introduction at $t_1 = 10,000$ years, the viruses from both species spread rapidly through the population, infecting most individuals and causing a marked decrease of the total population size (black line). A large fraction of hosts become immune against A viruses, and chronically infected with viruses of the B species (blue line). In contrast, only a few individuals are immune against the B species and chronically infected with A viruses (cyan line). Individuals chronically infected with both viral species (red line) decrease in time, whereas hosts able to clear both infections evolve (green line). (B) Haplotype composition in the NKR cluster at different time points (snapshots taken from the video provided in the [supplementary material, Supplementary Material](#) online). Each haplotype is represented by one dot, the size of which illustrates the frequency of the haplotype in the population. The position of each dot represents the number of iNKRs and aNKRs in the haplotype. In the lower triangle of each box, every haplotype is colored according to the average MHC coverage of its iNKRs. The MHC coverage is defined as the probability for an NKR to recognize any MHC molecule in the population. The degrees of MHC coverage are shown in the color bar from lowest (red) to highest (pink). The diagonal lines represent the maximal number of genes per haplotype we allow in our simulations. Mirrored on the diagonal, the haplotypes are colored according to the MHC coverage of the aNKRs. At $t_{\text{end}} = 3,000,000$, there are nine common haplotypes as estimated by the SRI.

express molecules that are similar to the MHC molecules in the population (fig. 4D), that is, viruses evolve MHC-mimics. The evolutionary advantage of MHC-like molecules is large, as MHC-mimics hamper viral detection by aNKRs, and impede iNKRs to detect missing self and provide protection against an MHC downregulating virus. Even after the evolution of more specific NKRs, hosts cannot fully adapt to the MHC-mimics. At the end of the simulations, the iNKRs are “fooled” by the MHC-like molecule in approximately 30% of the infections (fig. 3C; sum of protection by aNKRs and none). Thus, self/nonself discrimination becomes more challenging when viruses evolve MHC-mimics.

Viral species A and B consistently evolve MHC-I like molecules in all 15 simulations, indicating that MHC-I mimics play a major role in their evasion of the NK cell immune responses, irrespective of the ability of the virus to downregulate MHC. We tested this by modeling host populations infected with

viruses that are unable to mutate. These hosts are also able to recover from the infections, yet with a different evolutionary strategy (supplementary fig. S3, [Supplementary Material](#) online). When viruses do not mutate, aNKRs can become specialized to particular viral molecules and hence evolve a high specificity. On the contrary, iNKRs evolve an intermediate specificity, which allows them to bind most of the MHC-I molecules in the population. Note that viruses downregulating MHC-I are less likely to “fool” iNKRs if their molecules cannot evolve to become MHC-like. Therefore, iNKRs do not need to be highly specific to distinguish self from nonself in this case. Because aNKRs become highly specific and iNKRs intermediate specific, it is much easier to have licensed receptors, resulting in the evolution of small haplotypes. As we obtain different results with nonmutating viruses, we conclude that viral evolution shapes the evolution of both specificity and polygenicity in the NKR cluster.

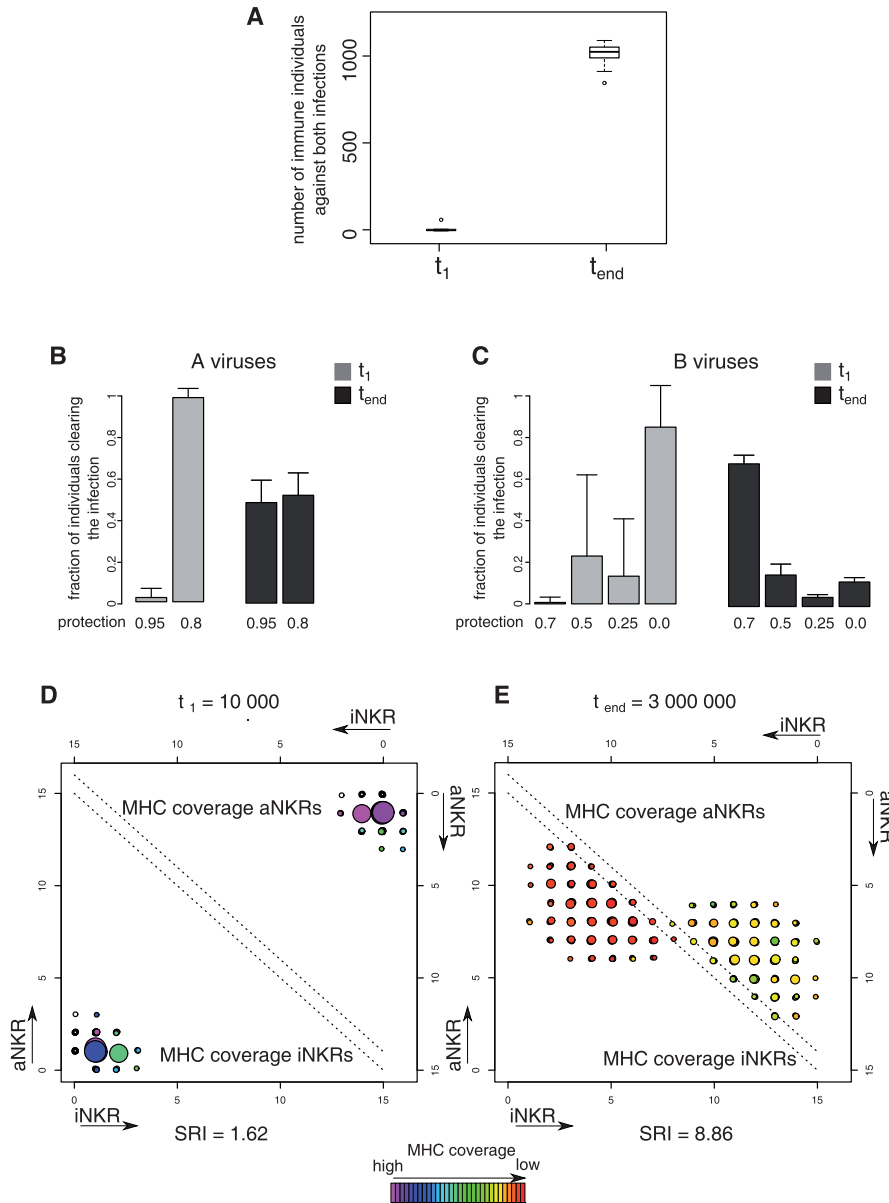


FIG. 3. Expansion of the NKR haplotypes leads to better immunity. (A) Individuals immune to both viral species at $t_1 = 10,000$ years and $t_{end} = 30,000,000$ years. (B) Observed fraction of hosts clearing A viruses with $P_{A1} = 0.95$, or $P_{A2} = 0.8$ at $t_1 = 10,000$ years and $t_{end} = 3,000,000$ years. (C) Observed fraction of hosts clearing B viruses with $P_{B3} = 0.7$, $P_{B1} = 0.5$, $P_{B4} = 0.25$, and $P_{B2} = 0$ at $t_1 = 10,000$ years and $t_{end} = 3,000,000$ years. At the beginning of the infection (t_1), A viruses tend to be cleared with $P_{A1} = 0.8$, whereas most individuals cannot clear B viruses and end up in the $P_{B2} = 0$ category. Consequently, the number of hosts immune against both viral species is close to zero. At the end of the simulation (t_{end}), the fraction of individuals with a high level of protection against B species increases to approximately 50%, resulting in an increase in the number of double immune individuals. The boxplots and the barplots show the mean out of 15 simulations. NKR haplotypes from all 15 simulations at t_1 are shown in (D) and t_{end} in (E). The composition and specificity are as described in figure 2B. At t_{end} , there are on average nine common haplotypes per simulation as calculated by the SRI (several symbols are overlapping).

Taken together, we show that it is beneficial for viruses to evolve MHC-mimics, and that populations adapt to such viruses by evolving expanded NKR clusters composed of several specific activating and inhibiting receptors.

Discussion

The exact evolutionary advantage of the polygenicity and polymorphism within the NKR cluster has remained puzzling. As the detection of “missing-self” can be achieved by monomorphic and conserved receptor ligand-interactions, the

evolution of polymorphic iNKR genes seems redundant. Even more intriguing is the unknown role of aNKRs, for which most of the ligands have not been identified yet. In this work, we study the evolution of NKRs in host populations infected with different viral species. We find that viruses have a large evolutionary advantage when they evolve MHC-like molecules, especially if they are capable of downregulating the expression of MHC-I on infected hosts. In turn, hosts evolve expanded haplotypes composed of various specific aNKRs, and iNKRs that are specialized on particular MHC molecules.

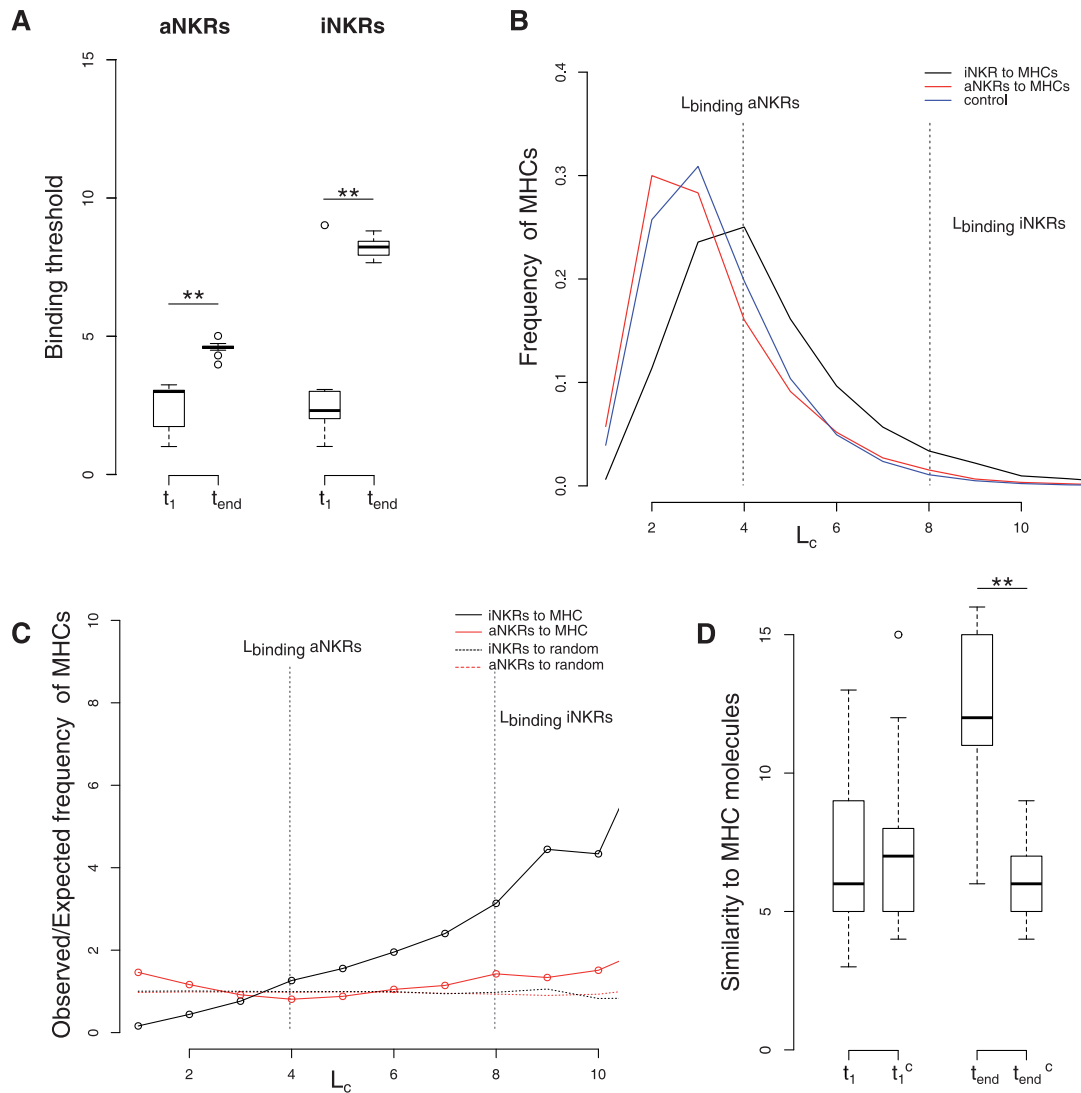


FIG. 4. Evolution of NKR specificity binding when viruses evolve MHC-I mimics. We study the evolution of NKR specificity by comparing the binding threshold of iNKRs and aNKRs (A) at the beginning of the infection ($t_1 = 10,000$ years) and at the end of the simulation ($t_{end} = 3$ My). (B) Fraction of MHC molecules that are recognized with a particular complementary adjacent length L_c . The blue curve depicts the expected frequency distribution determined by measuring the L_c between a 1,000 random NKRs and a pool of 30 MHC alleles, and repeating this experiment 1,000 times. The red and black curves represent the frequency distribution of the evolved aNKRs and iNKRs, respectively, at t_{end} . In both cases, the frequency distribution was determined by measuring the L_c of the evolved NKRs to the MHC alleles in their own population, and averaging over 15 simulations. (C) The fold change between the observed and expected frequency of MHC molecules recognized with a given L_c of the evolved iNKRs (black) and aNKRs (red). There is no difference to the expected values when analyzing the evolved NKRs to random MHC molecules (dashed lines). (D) Similarity between the viral and MHC molecules, determined by the maximal adjacent match between their bit strings. The boxplots represent the average over 15 simulations. t_x^c describes the control analysis we use at the different time points. As a control, we use randomly generated MHC molecules in (A) and randomly generated viral molecules in (D). In (A)–(D), the boxes represent the interquartile range, and the thick horizontal lines the median out of 15 simulations (** P values < 0.005 , and were calculated using the Mann–Whitney U test).

Thus, a polygenic and polymorphic NKR cluster encoding for specific receptors is beneficial to cope with coevolving pathogens.

To model this highly complex evolutionary process, simplifying assumptions were necessary. As discussed in detail in (Carrillo-Bustamante et al. 2013, 2014), our ABM is inspired by humans and KIRs, which has the advantage of having realistic parameters for processes such as birth and death. However, by changing parameters, the ABM can be adapted to other species, as it qualitatively represents a model of the evolution

of the expansion of the NKR complex. Indeed, our results hold true for different parameter settings and most of our assumptions (see Materials and Methods and [supplementary fig. S4](#), [Supplementary Material](#) online). We have focused on modeling only the evolution of NKRs, while fixing MHC polymorphism, despite the fact that these systems coevolve (Sambrook et al. 2005; Parham and Moffett 2013). Given that MHC-I alleles are evolutionary older than both Ly49 and KIR alleles, we chose to model the expansion and contraction of NKRs within an already existing MHC

Table 1. Level of Protection of the Host Depends on the Interactions between Its NKR and the Viral Molecule.

	At Least One iNKR Binds the Viral Molecule	At Least One aNKR Binds the Viral Molecule	Outcome	Host's Protection Level
Virus A	No ^a	No	The infection is cleared by T cells only	$P_{A1} = 0.8$
	No ^a	Yes	The infection is cleared by T cells and NK cells	$P_{A2} = 0.95$
Virus B	No	No	All NK cells in the host can detect missing-self	$P_{B1} = 0.5$
	Yes	No	Some subsets of NK cells will not detect missing-self (i.e., they will be "fooled" by the viral molecule)	$P_{B2} = 0.0$
	No	Yes	All NK cells in the host can detect missing self. Additionally, some NK cells will have an activating signal	$P_{B3} = 0.7$
	Yes	Yes	Some NK cells will be "fooled," some NK cells will have an activating signal	$P_{B4} = 0.25$

^aiNKRs cannot be engaged by the viral molecule during an infection with A viruses, as they are always engaged by the host's MHC molecules.

polymorphism. This choice has also practical reasons, as it is difficult to keep MHC polymorphism without including T-cell responses (Borghans et al. 2004). We speculate that, if we were able to enable coevolution of MHC-I molecules, these would evolve some structure, enabling them to be recognized by the NKRs (e.g., similar to the HLA-A3/A11, Bw4, C1, and C2 epitopes that are known to be primary KIR ligands). The evolution of structured MHC-I ligands would in turn affect the evolution of NKR specificity and probably haplotype composition in a manner similar to what is reported here. Thus, we expect no large qualitative changes, as specificity (and hence diversity) remains necessary to successfully detect self from nonself.

Probably, the most critical assumption for our results is the asymmetry in the protection levels, as we assume that "at least one" aNKR, but "none" of the iNKRs should bind the viral molecule, to protect the host. We based this asymmetry on experimental data showing susceptibility and protection of mice to MCMV. C57BL/6 mice are resistant against MCMV because of their aNKR Ly49H binding the MCMV encoded protein m157 (Arase et al. 2002; Smith et al. 2002), indicating that one aNKR directly recognizing decoy molecules is sufficient to provide protection. Similarly, the susceptibility of 129/J mice has been related to their iNKR Ly49I binding m157 with high affinity (Smith et al. 2002). This observation suggests that the contribution of one single inhibiting receptor dominates, making the mice susceptible. The latter is surprising because those NK cell subsets that do not express iNKRs recognizing the decoy should be able to detect missing self, proliferate, and protect its host. Whether Ly49I is the only iNKR binding m157 remains unknown, but the data clearly demonstrate that one iNKR-m157 interaction is sufficient for the virus to evade immunosurveillance.

The spontaneous evolution of MHC-like molecules by both types of viruses in our model suggests that this evasion mechanism provides an evolutionary advantage for many viruses. A virus expressing MHC-mimics can avoid missing-self detection by engaging iNKRs, and will escape from aNKR-mediated NK cell responses as aNKRs should have low affinity for cognate MHC molecules. The few examples of CMV-encoded MHC-I-like molecules (including m157, and m04

in mice, and UL18 in humans) are in line with our findings. Given that MHC-like molecules evolve so easily in our model because of their evolutionary advantage, it remains intriguing why only few viral MHC mimics have been found in other viral species, but it is possible that these molecular mimics are encoded by short sequences, and are thereby difficult to identify. It has been suggested that NK cells play an important role during infection with persistent pathogens (such as CMV and other herpesviruses), as these viruses require constant vigilance (Sun and Lanier 2009). Herpesviruses and poxviruses are large DNA viruses that employ many mechanisms to escape from the immune response. Several studies have successfully elucidated many of the immune evasion strategies used by EBV and CMV (Hassink et al. 2005; van Gent, Braem, et al. 2014; van Gent, Gram, et al. 2014) by eliminating the expression of individual viral genes from infected cells and studying the effect of these viral mutants on the immune response. However, our current understanding of the immunoevasive mechanisms employed by these large DNA viruses is far from complete, and we would predict that these viruses encode more MHC-mimicking molecules than currently known. Extending this type of functional screening (Hassink et al. 2005; van Gent, Braem, et al. 2014; van Gent, Gram, et al. 2014) to large number of viral proteins, and specifically assessing the effect of mutant viruses on NK cell responses, could confirm our predictions.

The characterization of specific ligands for aNKRs has remained difficult. Even though some specific interactions (e.g., Ly49H/m157, or Ly19P/m04-H2^k) have been identified, the ligands for most activating KIRs remain unknown. Our model shows that it is indeed difficult to evolve specific interactions between aNKRs and particular viral proteins as viruses evolve rapidly, and become a moving target for aNKRs, impeding the adaptation to particular infectious pathogens. Note that the specific interactions found in the mouse CMV model are from particular inbred mice strains infected with one specific laboratory CMV strain. In outbred wild mice, Ly49H-mediated resistance is rather uncommon (Scalzo et al. 2005), confirming that there is large heterogeneity in the interactions between aNKRs and viruses.

Although iNKR and aNKR share a large sequence similarity in their extracellular binding domain, aNKRs do not bind the ligands of iNKRs (Saulquin et al. 2003; Gillespie et al. 2007; O'Connor and McVicar 2013). Studies have even shown that there is an evolutionary trend in humans and chimpanzees toward reducing the affinity of the aNKRs to the HLA epitopes C1 and C2 (Moesta et al. 2010). On the contrary, inhibitory KIRs have rather specific interactions, binding preferentially four epitopes on HLA molecules (A3/11, Bw4, C1 and C2) (Moretta et al. 1996). We show that the specialization of iNKRs but not aNKRs on a small group of MHC-I molecules is advantageous to discriminate self/nonself more efficiently. However, the required specificity predicted by our model is higher than the known specificity for just four HLA-epitopes. Although binding experiments of KIR-Fc fusions to a broad panel of HLA molecules have revealed the specificity of KIR2DL2 and KIR2DL3 (Moesta et al. 2008, 2010), an extension of these studies to a larger set of KIR alleles (including all inhibiting and activating KIRs) would reveal how many MHC-I molecules an NKR can recognize, and would establish the specificity of NKRs in a quantitative manner.

Summarizing the evolution of both host receptors and viral immunomodulatory molecules is an ongoing dynamic process. We have shown that the NKR genetic complex evolves polygenicity and specificity in response to rapidly coevolving viruses.

Materials and Methods

Agent-Based Model

The ABM consists of two types of agents, that is, hosts and viruses, and three types of events: Birth, death, and infection. During each time step of 1 week, we screen all hosts in a random order and confront them to one of the events. Hosts age over time and their ages, infection states, and infection types are updated at the end of every time step. This cycle is repeated for 3 My to simulate long-term evolution. The model used here is based on our previously published ABMs (Carrillo-Bustamante et al. 2013, 2014). Below is a detailed description of the extensions we implemented in the current model. The full description and model parameters are described in (Carrillo-Bustamante et al. 2013, 2014).

Bitstrings

All molecules, that is, NKRs, MHC-I, and viral proteins, are modeled with bit strings of length $L = 16$. Two molecules can interact whenever the longest adjacent complementary match between their strings exceeds a binding threshold L_{binding} (supplementary fig. S1A, Supplementary Material online).

We create a gene pool of 30 MHC alleles which reflects the most common HLA B, and C alleles in the European population (Meyer et al. 2007). The MHC molecules are not completely random, and they have a hamming distance (HD) of maximal 4 bits among each other. To create these alleles, we first create one random bit string and generate all possible strings that have an HD = 1 to the original sequence. For each of these “first generation mutants,” we generate again all

possible strings that have an HD = 1. This ensures that the HD is maximally 4. We randomly select 30 strings out of these “second-generation mutants” to fill the MHC pool.

Mutations

Only NKRs and viral molecules are allowed to mutate in our model. During a mutation event, NKRs evolve their bit string, binding threshold, and signaling type. That is, a new bit string will be randomly generated, to which a random L_{binding} will be assigned. This model approach has the advantage of reducing computational time, without affecting the main outcome of the simulations (supplementary fig. S4C and D, Supplementary Material online). This new NKR can be inhibiting or activating with equal probability of 0.5. We do not model point mutations in order to decrease computational time. Similarly, viral molecules mutate into a new random bit string with a small probability ($P = 0.0005$) upon transmission to a new host.

NK Cell Education

We establish the host's repertoire of licensed NKRs at birth. iNKRs become licensed if they recognize at least one of the MHC molecules in its host. aNKRs become licensed if they do not bind any of the host's MHC molecules within an individual. Additionally, an aNKR can only be licensed if that host has already one licensed iNKR (supplementary fig. S1, Supplementary Material online).

Viral Infections

In these simulations, we consider two viral species. Both species, A and B, express a viral-encoded molecule, representing viral proteins that are expressed on infected cells. The B species downregulates the MHC expression in the infected host, which allows us to model viruses such as CMV, or HIV that use this mechanism to escape T-cell responses (Collins et al. 1998; Gall et al. 1998; Schust et al. 1998; Cohen et al. 1999; Gewurz et al. 2001; Llano et al. 2003). Viral molecules are randomly generated at the beginning of the simulation, and each virus can express only one.

Protection Levels

Whether or not a host is protected against a viral infection depends on the interactions between its repertoire of licensed NKRs and the viral molecule. On the NKR repertoire level, there are two major outcomes: 1) At least one licensed iNKR binds the viral molecules and 2) at least one licensed aNKR binds the viral molecules (fig. 1). Based on these two outcomes, we can define different levels of protection for the host as described in detail in table 1. Viral strains from the A species do not downregulate MHC. Hence, they do not escape the adaptive immune response, and can therefore be cleared with a high probability $P_{A_1} = 0.8$. However, if the host has at least one licensed aNKR binding a viral molecule of the A viruses, there is an additional activation of NK cells and we increase the clearance probability to $P_{A_2} = 0.95$.

By downregulating the MHC expression, viruses from the B species escape the T-cell response, and have a higher fitness

than A viruses. Whether or not the host can clear the infection depends only on its repertoire of licensed NKRs. If none of the iNKRs binds the viral molecule, then all NK cells in that host will be able to detect missing-self, and we set $P_{B1} = 0.5$ (see table 1). If at least one licensed iNKR but no aNKR binds the viral molecule, a subset of NK cells in that host will be “fooled” by the virus. Experimental data suggest that in this case the host is not protected (Smith et al. 2002). As the virus escapes the response of both T and NK cells, we set $P_{B2} = 0.0$. If none of the host’s licensed iNKR binds the viral molecule and at least one aNKR does, then all NK cells will detect missing-self and some subsets will receive a strong activation signal, and we set $P_{B3} = 0.7$. We set this value lower than $P_{A1} = 0.8$ (see table 1) because the viruses from the B species always escape T-cell responses and should therefore have an advantage over the A strains. Finally, if at least one licensed iNKR and one aNKR bind a viral molecule, there are several possible NK cell subsets: Some NK cells will be “fooled,” whereas others will receive an activating signal. We define a low level of protection by setting $P_{B4} = 0.25$, assuming that those NK cell subsets that are “fooled” dominate. Note that increasing P_{B4} does not affect the main result of our simulations (supplementary fig. S4B, Supplementary Material online).

Model Initialization

The model is initialized with a host population of 4,500 individuals, with random ages between 10 and 70 years. Hosts carry two MHC loci. Similarly, the initial NKR cluster is composed of two loci, encoding for one degenerate receptor pair, that is, one iNKR and one aNKR, each being able to recognize all MHCs in the population. Thus, initially none of the aNKRs will become licensed.

Model Parameters

We have set the infection rates and host survival rates such that both viruses and hosts are maintained during the epidemic (Carrillo-Bustamante et al. 2013, 2014). Other parameters such as birth, death, and mutation rates are adopted from a human population (Carnes et al. 2006). As our main results are of a qualitative nature, they hardly depend on the parameter values. The critical parameters in our model are the probabilities of clearing the infection, that is, P_A and P_B , as they determine the disease outcome and with it the selection pressure each virus imposes on the hosts. Changing these values does not affect qualitatively our results, as shown in supplementary figure S4A and B, Supplementary Material online.

Analysis of Recognized MHC Molecules

To determine the expected distribution of the maximal complementary match L_c , we generate 1,000 random NKRs and measure their longest complementary adjacent match to 30 randomly generated MHC alleles. The frequency of recognized MHCs with a particular L_c is averaged over 1,000 of these “experiments.” We also perform the analysis using 30 “structured” MHC alleles, that is, alleles generated with a

maximal HD = 4 as explained above; however, the distribution of expected maximal complementary match remains the same (results not shown)

To analyze how this distribution changes after evolution, we measure the longest complementary adjacent matches between NKRs and the MHC molecules at $t_{\text{end}} = 3$ My. The final distribution is the average out of 15 simulations.

We also analyze the distribution of L_c between the evolved NKRs and random MHC molecules by measuring the L_c of an each NKR to 30 random MHC alleles, and repeating this experiment 1,000 times. Here too, the frequency distribution does not deviate from the expected values (results not shown).

Genetic Diversity

The Simpson’s Index is a measurement of diversity that can be interpreted as the probability that two randomly chosen haplotypes from two random hosts in the population are identical (Simpson 1949). The reciprocal of the Simpson’s Index defines a “weighed” diversity. The SRI was calculated as follows: $SRI = \frac{1}{\sum_{i=1}^N f_i^2}$, where f_i is the frequency of the haplotype i over all NKRs in the population, and N is the total number of unique NKRs.

Supplementary Material

Supplementary material and figures S1–S4 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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References

- Abi-Rached L, Parham P. 2005. Natural selection drives recurrent formation of activating killer cell immunoglobulin-like receptor and Ly49 from inhibitory homologues. *J Exp Med.* 201:1319–1332.
- Anfossi N, André P, Guia S, Falk CS, Roetynck S, et al. 2006. Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 25:331–342.
- Arase H, Lanier LL. 2002. Virus-driven evolution of natural killer cell receptors. *Microbes Infect.* 4:1505–1512.
- Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 296:1323–1326.
- Averdam A, Petersen B, Rosner C, Neff J, Roos C, et al. 2009. A novel system of polymorphic and diverse NK cell receptors in primates. *PLoS Genet.* 5:e1000688.
- Bacon L, Eagle RA, Meyer M, Easom N, Young NT, et al. 2004. Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D. *J Immunol.* 173:1078–1084.
- Bauer S, Groh V, Wu J, Steinle A, Phillips JH, et al. 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285:727–729.
- Borghans JAM, Beltman JB, Boer RJD. 2004. MHC polymorphism under host-pathogen coevolution. *Immunogenetics* 55:732–739.

- Borrego F, Ulbrecht M, Weiss EH, Coligan JE, Brooks AG. 1998. Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J Exp Med*. 187:813–818.
- Braud VM, Allan D, O'Callaghan CA, Söderström K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, et al. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391:795–799.
- Carnes BA, Holden LR, Olshansky SJ, Witten MT, Siegel JS. 2006. Mortality partitions and their relevance to research on senescence. *Biogerontology* 7:183–198.
- Carrillo-Bustamante P, Keşmir C, de Boer RJ. 2013. Virus encoded MHC-like decoys diversify the inhibitory KIR repertoire. *PLoS Comput Biol*. 9:e1003264.
- Carrillo-Bustamante P, Keşmir C, de Boer RJ. 2014. Quantifying the protection of activating and inhibiting NK cell receptors during infection with a CMV-like virus. *Front Immunol*. 5:20.
- Cerwenka A, Bakker AB, McClanahan T, Wagner J, Wu J, et al. 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 12:721–727.
- Cerwenka A, Lanier LL. 2001. Ligands for natural killer cell receptors: redundancy or specificity. *Immunol Rev*. 181:158–169.
- Chalifour A, Scarpellino L, Back J, Brodin P, Devèvre E, et al. 2009. A role for cis interaction between the inhibitory Ly49A receptor and MHC class I for natural killer cell education. *Immunity* 30:337–347.
- Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, et al. 1999. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* 10:661–671.
- Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. 1998. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 391:397–401.
- Elliott JM, Yokoyama WM. 2011. Unifying concepts of MHC-dependent natural killer cell education. *Trends Immunol*. 32:364–372.
- Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaëlsson J. 2010. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood* 115:1166–1174.
- Gall SL, Erdtmann L, Benichou S, Berlioz-Torrent C, Liu L, et al. 1998. Nef interacts with the mu subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. *Immunity* 8:483–495.
- Gewurz BE, Wang EW, Tortorella D, Schust DJ, Ploegh HL. 2001. Human cytomegalovirus US2 endoplasmic reticulum-lumenal domain dictates association with major histocompatibility complex class I in a locus-specific manner. *J Virol*. 75:5197–5204.
- Gillespie GMA, Bashirova A, Dong T, McVicar DW, Rowland-Jones SL, et al. 2007. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8+ T cell epitopes. *AIDS Res Hum Retroviruses*. 23:451–455.
- Guethlein LA, Older Aguilar AM, Abi-Rached L, Parham P. 2007. Evolution of killer cell Ig-like receptor (KIR) genes: definition of an orangutan KIR haplotype reveals expansion of lineage III KIR associated with the emergence of MHC-C. *J Immunol*. 179:491–504.
- Hassink GC, van Dam JGD, Koppers-Lalic D, van Gaans-van den Brink J, van Leeuwen D, et al. 2005. Rat cytomegalovirus induces a temporal downregulation of major histocompatibility complex class I cell surface expression. *Viral Immunol*. 18:607–615.
- Kielczewska A, Pyzik M, Sun T, Krmpotic A, Lodoen MB, et al. 2009. Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response. *J Exp Med*. 206:515–523.
- Lanier LL. 2005. NK cell recognition. *Annu Rev Immunol*. 23:225–274.
- Lanier LL. 2008. Evolutionary struggles between NK cells and viruses. *Nat Rev Immunol*. 8:259–268.
- Lee SH, Girard S, Macina D, Busà M, Zafer A, et al. 2001. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat Genet*. 28:42–45.
- Llano M, Gumá M, Ortega M, Angulo A, López-Botet M. 2003. Differential effects of US2, US6 and US11 human cytomegalovirus proteins on HLA class Ia and HLA-E expression: impact on target susceptibility to NK cell subsets. *Eur J Immunol*. 33:2744–2754.
- López-Vázquez A, Rodrigo L, Martínez-Borra J, Pérez R, Rodríguez M, et al. 2005. Protective effect of the HLA-Bw4180 epitope and the killer cell immunoglobulin-like receptor 3DS1 gene against the development of hepatocellular carcinoma in patients with hepatitis C virus infection. *J Infect Dis*. 192:162–165.
- Martin MP, Gao X, Lee JH, Nelson GW, Detels R, et al. 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet*. 31:429–434.
- Meyer D, Singe R, Mack S, Lancaster A, Nelson M, et al. 2007. Single locus polymorphism of classical HLA genes. In: Hansen JA, editor. Immunobiology of the human MHC: Proceedings of the 13th International Histocompatibility Workshop and Conference. Vol. 1. Seattle, WA: IHWG Press. p. 653–704.
- Moesta AK, Graef T, Abi-Rached L, Older Aguilar AM, Guethlein LA, et al. 2010. Humans differ from other hominids in lacking an activating NK cell receptor that recognizes the C1 epitope of MHC class I. *J Immunol*. 185:4233–4237.
- Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, et al. 2008. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. *J Immunol*. 180:3969–3979.
- Moretta A, Bottino C, Vitale M, Pende D, Biassoni R, et al. 1996. Receptors for HLA class-I molecules in human natural killer cells. *Annu Rev Immunol*. 14:619–648.
- O'Connor GM, McVicar D. 2013. The yin-yang of KIR3DL1/S1: molecular mechanisms and cellular function. *Crit Rev Immunol*. 33:203–218.
- Parham P, Abi-Rached L, Matevosyan L, Moesta AK, Norman PJ, et al. 2010. Primate-specific regulation of natural killer cells. *J Med Primatol*. 39:194–212.
- Parham P, Moffett A. 2013. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat Rev Immunol*. 13:133–144.
- Pelak K, Need AC, Fellay J, Shianna KV, Feng S, et al. 2011. Copy number variation of KIR genes influences HIV-1 control. *PLoS Biol*. 9:e1001208.
- Petrie EJ, Clements CS, Lin J, Sullivan LC, Johnson D, et al. 2008. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J Exp Med*. 205:725–735.
- Pyzik M, Charbonneau B, Gendron-Pontbriand EM, Babić M, Krmpotic A, et al. 2011. Distinct MHC class I-dependent NK cell-activating receptors control cytomegalovirus infection in different mouse strains. *J Exp Med*. 208:1105–1117.
- Sambrook JG, Bashirova A, Palmer S, Sims S, Trowsdale J, et al. 2005. Single haplotype analysis demonstrates rapid evolution of the killer immunoglobulin-like receptor (KIR) loci in primates. *Genome Res*. 15:25–35.
- Saulquin X, Gastinel LN, Vivier E. 2003. Crystal structure of the human natural killer cell activating receptor KIR2DS2 (CD158j). *J Exp Med*. 197:933–938.
- Scalzo AA, Manzur M, Forbes CA, Brown MG, Shellam GR. 2005. NK gene complex haplotype variability and host resistance alleles to murine cytomegalovirus in wild mouse populations. *Immunol Cell Biol*. 83:144–149.
- Schust DJ, Tortorella D, Seebach J, Phan C, Ploegh HL. 1998. Trophoblast class I major histocompatibility complex (MHC) products are resistant to rapid degradation imposed by the human cytomegalovirus (HCMV) gene products US2 and US11. *J Exp Med*. 188:497–503.
- Simpson E. 1949. Measurement of diversity. *Nature* 163:688.
- Smith HRC, Heusel JW, Mehta IK, Körner BG, Naidenko OV, et al. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci U S A*. 99:8826–8831.
- Sun JC, Lanier LL. 2008. Tolerance of NK cells encountering their viral ligand during development. *J Exp Med*. 205:1819–1828.

- Sun JC, Lanier LL. 2009. The natural selection of herpesviruses and virus-specific NK cell receptors. *Viruses* 1:362.
- van Gent M, Braem SGE, de Jong A, Delagic N, Peeters JGC, et al. 2014. Epstein-Barr virus large tegument protein BPLF1 contributes to innate immune evasion through interference with toll-like receptor signaling. *PLoS Pathog.* 10:e1003960.
- van Gent M, Gram AM, Boer IGJ, Geerdink RJ, Lindenbergh MFS, et al. 2014. Silencing the shutoff protein of Epstein-Barr virus in productively infected B cells points to (innate) targets for immune evasion. *J Gen Virol.*
- Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. 2008. Functions of natural killer cells. *Nat Immunol.* 9(5):503–510.
- Voigt V, Forbes CA, Tonkin JN, Degli-Esposti MA, Smith HRC, et al. 2003. Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells. *Proc Natl Acad Sci U S A.* 100:13483–13488.
- Wilhelm BT, Gagnier L, Mager DL. 2002. Sequence analysis of the ly49 cluster in C57BL/6 mice: a rapidly evolving multigene family in the immune system. *Genomics* 80:646–661.
- Wilhelm BT, Mager DL. 2004. Rapid expansion of the Ly49 gene cluster in rat. *Genomics* 84:218–221.
- Zeng L, Sullivan LC, Vivian JP, Walpole NG, Harpur CM, et al. 2012. A structural basis for antigen presentation by the MHC class Ib molecule, Qa-1b. *J Immunol.* 188:302–310.