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Phenotypic differences in viral immune escape explained by linking within-host dynamics to host-population immunity

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Abstract

Viruses that do not cause life-long immunity persist by evolving rapidly in response to prevailing host immunity. The immune-escape mutants emerge frequently, displacing or co-circulating with native strains even though mutations conferring immune evasion are often detrimental to viral replication. The epidemiological dynamics of immune-escape in acute-infection viruses with high transmissibility have been interpreted mainly through immunity dynamics at the host population level, despite the fact that immune-escape evolution involves dynamical processes that feedback across the within- and between-host scales. To address this gap, we use a nested model of within- and between-host infection dynamics to examine how the interaction of viral replication rate and cross-immunity imprint host population immunity, which in turn determines viral immune escape. Our explicit consideration of direct and immune-mediated competitive interactions between strains within-hosts revealed three insights pertaining to risk and control of viral immune-escape: (1) replication rate and immune-stimulation deficiencies (i.e., original antigenic sin) act

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Appendix A. Supporting information

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synergistically to increase immune escape, (2) immune-escape mutants with replication deficiencies relative to their wildtype progenitor are most successful under moderate cross-immunity and frequent re-infections, and (3) the immunity profile along short host-transmission chains (local host-network structure) is a key determinant of immune escape.

Keywords

Cross-immunity; Within-host dynamics; Original antigenic sin; Adaptive immunity; Competition

1. Introduction

Selection by host immunity, whether natural or artificially induced, is a major driver of viral evolution. Host immunity imposes selection on viruses at two linked scales: replication within hosts and transmission/establishment in subsequent hosts. Immune responses *within* hosts impact transmissibility through affecting the time course of viral abundance; moreover, levels of immunity in the host *population* modify patterns of transmission by determining establishment of infection and hence the length and structure of host-to-host transmission chains. A mechanistic understanding of the interdependence of immunity structure and viral evolution across scales is thus key for understanding disease-emergence risk and efficacy of viral disease-intervention methods.

In addition to host immunity, intrinsic genetic constraints in the viral genome govern immune escape. Phenotypic studies of immune-escape mutants show that these mutations are often pleiotropic, causing reduced replication rates (Berkhoff et al., 2006; Novella et al., 2005; Rimmelzwaan et al., 2005; Rudneva et al., 2005). This reduction in replication can impose a significant fitness trade-off for the virus, since experimental adaptation studies show that immune-escape strains acquire compensatory mutations when the specific immune pressure is removed (Rimmelzwaan et al., 2005; Rudneva et al., 2005), and wildtype strains often re-emerge several seasons after emergence of immune-escape strains (Berkhoff et al., 2007; Boon et al., 2002). However, immune-escape mutants with replication deficiencies can arise and predominate in a wildtype population extremely rapidly during seasonal epidemics of influenza, even when the margin of advantage from immune escape is thought to be small (Berkhoff et al., 2007; Voeten et al., 2000). What conditions explain this counter-intuitively high rate of immune-escape evolution?

Epidemiological models of immune escape in acute-infections (e.g., influenza viruses, noroviruses, rotaviruses) have identified how individual factors that govern the rate of escape operate; from viral life-history traits such as replication rates or infectious periods, to immune-mediated processes such as cross-immunity and recovery rates, to population processes such as epidemic size and herd immunity (Boni et al., 2006; Gog, 2008; Gog et al., 2003; Nuno et al., 2007; Park et al., 2009; Recker et al., 2007). However, these types of population models do not explicitly account for the effects of immunity on transmissibility throughout infection (i.e., time-varying transmissibility), thus separating the scales at which selection operates. Recently, nested models of host-parasite evolutionary dynamics, which link within- and between-host dynamics, have been developed to address this deficit (Alizon

and van Baalen, 2008; Andre and Gandon, 2006; Coombs et al., 2007; Ganusov and Antia, 2006b; Ganusov et al., 2002; Gilchrist and Sasaki, 2002; Lange and Ferguson, 2009; Luciani and Alizon, 2009; Mideo et al., 2008; Read and Keeling, 2006). These models have derived fitness trade-offs that arise between within-host parameters and host responses, and shown that linkage of within- and between-host selection predicts different evolutionary dynamics than when these scales are considered separately. Thus, nested models provide an essential mechanistic foundation for examining the evolutionary epidemiology of viral immune escape.

Two recent studies emphasize the utility of nested models for understanding viral immune escape. Lange and Ferguson (2009) show how different host-contact rates and contact-network structures predict different between-host fitness optima that can be reached through selection on viral replication rate (i.e., a within-host viral parameter). Luciani and Alizon (2009), on the other hand, address how between-host fitness guides evolution of within-host viral replication, showing that optimal replication rates are affected by mutation rates and cross-immunity. Both studies reveal that viral replication rate evolves differently depending on between-host fitness optima. However, neither study investigates how the within-host dynamics change the profile of immunity in the host population, which alters immune-selection pressure. Furthermore, previous nested models have focused on invasion fitness rather than how fitness may change as the host-population immunity profile changes. In order to predict immune-escape evolution in acute viral infections, several processes remain to be examined mechanistically in more detail: (1) how within-host interactions such as direct competition between strains and cross-immunity impact the immunity profile in the host population, (2) how the profile of host-population immunity modifies host-to-host transmission chains, and (3) how previous infection dynamics determine selection of immune-escape phenotypes.

We used a nested model to examine how the reciprocal influences of viral phenotype and host immune response impact selection of an immune-escape mutant. Our two-strain within-host model specifies viral phenotypes according to two parameters: replication rate and cross-immunity. We begin by identifying how replication rates and cross-immunity affect the within-host viral and immune effector dynamics and transmissibility. We then investigate how viral-induced immunity structure in the host population impacts the evolutionary trajectory of an immune-escape phenotype by comparing host transmission chains with arbitrarily determined immunity to those with immune memory (i.e., from previous infection dynamics). By linking within-host dynamics, transmission and immune memory, we found that a mutant with a moderate replication deficiency can have a selective advantage in the host population. We show how host-population immunity, which is the selective force behind immune-escape evolution, critically depends on within-host dynamics and interactions between particular viral phenotypes.

2. Methods

2.1 Conceptual overview

We consider the case of an immune-escape mutant arising in a host infected with wildtype (i.e., the mutant has a numerical disadvantage relative to its wildtype progenitor initially).

We use a simple, 'predator–prey' model of within-host immunity and viral dynamics and examine the trajectory of an immune-escape mutant that is introduced at the beginning of simulations in order to focus on the interdependencies of viral phenotype and the changing profile of host-population immunity (rather than stochastic effects of mutation). We also simplify transmission to a linear chain of hosts, where each host contacts and transmits to the next host only once, in order to focus on how immunity structure of the chain affects the rate of immune escape.

Within-host model—We considered two viral strains, wildtype (W) and evader (E), which are defined by replication rate (ρ) and cross-immunity (ε) parameters. The abundance of each strain is affected by two populations of immune effectors: primary (effectors most specific to self) and cross (effectors that are more specific to the other strain). The within-host dynamics were modeled by the following system of ODEs (which is an extension of the one-strain model presented by Gilchrist and Sasaki, 2002 with the addition of an explicit carrying capacity on total viral load):

$$\dot{V}_W = \rho_W V_W (1 - V_W - V_E) - V_W (I_W + \varepsilon_W I_E) \quad (1)$$

$$\dot{I}_W = I_W (V_W + \varepsilon_E V_E) \quad (2)$$

$$\dot{V}_E = \rho_E V_E (1 - V_E - V_W) - V_E (I_E + \varepsilon_E I_W) \quad (3)$$

$$\dot{I}_E = I_E (V_E + \varepsilon_W V_W) \quad (4)$$

V and *I* are the virus and immune effectors, respectively, and subscripts distinguish the two strains and immune-preference to each strain. Viral abundance is limited by a total maximum abundance, *K*, that is common to both strains. In order to reduce the number of free parameters, we re-scaled the variables to units of the carrying capacity, *K* (i.e., original form in the logistic population growth model: $\rho V_i (1 - (V_i + V_j)/K)$), and re-scaled the times by the inverse of the carrying capacity, resulting in the effective carrying capacity of 1 shown in Eqs. (1) and (3). Note that viral load can exceed the value of 1 only due to initial conditions, in which case it would decrease rapidly to < 1. Strains respond similarly to their primary effectors but recognition of E by W-primary effectors is decreased relative to the opposite by a factor ε (i.e., immune-escape; where $\varepsilon_E < \varepsilon_W$). Likewise, immune effectors are produced in proportion to both viral load of their primary targets and viral load of their cross-reactive targets (with decreased efficiency to non-primary virus). Thus, our within-host model includes three types of strain interactions that are known to impact viral and immunity dynamics within hosts: (1) direct competition (Pepin and Hanley, 2008; Shinjoh et al., 2000; Zwart et al., 2009) *via* a common maximum viral load, (2) immune- mediated competition *via* cross-immunity killing, and (3) original antigenic sin (OAS) (Kim et al., 2009) *via* cross-stimulation of immunity. OAS refers to the propensity of the immune system to stimulate production of immune effectors that cross-react with antigen, although less specifically, resulting in less efficient clearance than would be possible if primary

effectors were present. OAS has recently been demonstrated between strains of influenza virus (Kim et al., 2009), and effects of the cross-stimulation were found to be asymmetric, making it difficult to generalize the degree to which antigenic sin could enhance the rate of escape at the host population level. Effects of a replication rate were examined through the replication rate parameters: ρ_E and ρ_W.

2.3 Host contact structure and immunity in linear transmission chains

Host contact was simplified to a linear chain of hosts where each could potentially get infected from exactly one other. Although this contact structure is unrealistic, we made this simplification to isolate transmission effects of immunity structure from those of contact-network structure and multiple transmissions which we examine in subsequent work. Three types of immunity structure were investigated: (1) homogenous, where all individuals had the same initial immunity levels, (2) random heterogeneity, where all individuals had a random initial level of immunity ($I_0 \sim$ Uniform[0.001, 1]), and (3) memory heterogeneity, where individuals had the immunity levels from their previous infection. The third type of immunity involved re- infection of the same host. This type of heterogeneity in host transmission chains, which depends on the viral dynamics in previous infections, crucially affects the probability of immune escape (Recker et al., 2007), but has yet to be examined within a framework that integrates feedback between within-host and epidemiological dynamics. In the re-infection treatments, peak immunity levels from re-infections were re-scaled by a proportion (W) to allow for re-infection since the immunity variables did not have intrinsic carrying capacities (i.e., similar to immunity waning). Initial levels of immunity in naive hosts were arbitrarily designated as: $I_{OW} = I_{OE} = 0.001$ (i.e., I_{0i} must be non-zero) and were the lowest reference point for initial immunity. Experienced hosts were assumed to have prior exposure to W but not E (i.e., $I_{0W} > 0.001$ and $I_{0E} = 0.001$).

2.4 Transmission method 1: Peak load

In order to simplify the contact-transmission process to occur through a one-dimensional linear chain of hosts, it is necessary to estimate within-host fitness. We assumed the time of peak total viral load to be one surrogate of within-host fitness, since transmission probability is likely to be highest when total viral load is highest. Using this measure of within-host fitness, transmission was carried out by sampling viral strains in proportion to their abundance at the time point when *total* viral load was highest, taking into account both viruses during the entire infectious period. A constant proportion of the viral population (bottleneck) was used as the infectious dose for the subsequent infection. We implemented a minimum threshold on the infectious dose (10^{-7}) to allow for extinction since viruses are discrete entities and our within-host model is in continuous time.

2.5 Transmission method 2: Mean load

Since within-host fitness depends both on viral load and infectious period, our second estimate of within-host fitness was the mean load of each strain over the entire infectious period (i.e., when viral load was above a minimum threshold, 10^{-7}). Infectious dose at transmission was then the mean load of each strain multiplied by the bottleneck factor.

2.6 Transmission in a randomly mixing host population

To examine effects of the entire viral growth curve on transmission we simulated transmission in a randomly mixing population of 10,000 hosts. We compared transmission patterns under conditions where each host could only experience a single infection versus when re-infections were allowed. Re-infected hosts had the level of immunity that had arisen in the previous infection (no waning). Viral load during the entire infectious period was monitored for individual infected hosts and infection dose depended on viral load in the contact host at the time of transmission. As above, there was a minimum threshold on infectiousness (10^{-7}) and the dose that was transmitted was the current dose in the contact host multiplied by the bottleneck factor (10^{-2}). At each transmission time step, pairs of hosts were selected at random for contact. Transmission occurred if an infected host containing a viral load above the minimum infectious threshold after the bottleneck contacted an uninfected host. Transmission rate was a constant parameter (0.2) controlled by the proportion of hosts in the population that made contact at each transmission time step. Outputs were the number of new cases for each type of infection (W, E or co-infection), the infection history of hosts that were infected by the mutant (Naive, previous infection with W only, previous infection with E only or previous co-infection) and the mean levels of immunity present at recovery in hosts that recovered.

3. Results

3.1 Within-host viral dynamics

To examine the selection dynamics of an immune-escape mutant (E) arising through mutation in a wildtype (W) infection, we assumed that the mutant was initially rare, co-infected with wildtype and had moderate cross-immunity ($\varepsilon_W = 0.8$ and $\varepsilon_E = 0.28$). Fig. 1 compares the dynamics of E when inoculated at very low dose in single and co-infections. In single infections, E grows to a higher peak load at a faster rate relative to its replication in co-infections (Fig. 1, compare A and D and B and E). The magnitude of competitive suppression depended on replication rate relative to W (compare Fig. 1D and E) and the immune status of the host (compare Fig. 1D and E–G and H, respectively). In naive hosts, where initial immunity to both strains is very low (i.e., a level that is assumed to be negligible, see Section 2), the mutant with a replication rate advantage over wildtype (AE) can reach relatively high peak viral load despite its initial numerical disadvantage (Fig. 1D). On the other hand, the mutant with a replication deficiency (DE) is not able to replicate much before the strong immune response to W curtails its replication. In experienced hosts, which have elevated levels of W-primary effectors, both AE and DE can reach relatively high peak loads (Fig. 1G and H). However, DE does not surpass W, but has an extended infectious period, while AE does reach a higher peak load than W albeit with a shorter infectious period than DE. These dynamics predict that if transmission depends mainly on peak viral load that DE will be outcompeted in both naive and experienced hosts, whereas if transmission depends on mean viral load that DE could persist through transmission to experienced hosts.

3.2 Within-host immune response

The magnitude of response by primary effectors was affected by co-infection, replication rates and initial immunity. In co-infections of naive hosts, the mutant stimulated lower levels of primary effectors than in single infections (Fig. 1C, bars labeled 'N' versus Fig. 1F), and this effect (i.e., OAS, see Section 2) was amplified when the mutant had a replication deficiency. OAS was even stronger in experienced hosts due to the initially high levels of W-primary effectors that cross-react with E. The difference in magnitude of OAS between single and co-infections was lower when hosts were experienced then when they were naive (Fig. 1C, bars labeled 'E' versus Fig. 1I). In summary, the results predict that although both the DE and AE mutants can gain an advantage in re-infections of hosts that were initially experienced (through the under-stimulation of E-primary effectors), only DE would gain an advantage in re-infection of hosts that were initially naive.

3.3 Within-host advantage as a function of viral traits

Fig. 2 shows the influence of viral replication-rate and cross-immunity traits on within-host fitness and immune response. Regions where the mutant has an advantage in naive hosts by peak load (Fig. 2A and D), mean load (Fig. 2B and E), and under-stimulation of E-primary immunity (Fig. 2C and F) are indicated in gray. Note that the region of advantage in peak or mean load does not overlap with the region of advantage from OAS (Fig. 2A and B versus C), highlighting that effects of OAS are strongest when the mutant has a lower replication rate. Also, the magnitude of disadvantage in within-host fitness is lower in terms of mean load than peak load, suggesting that higher levels of immune escape would be predicted if mean load is used as proxy for fitness. In experienced hosts, the effect of OAS is similar in single and co-infections and the mutant can gain an advantage in within-host fitness over a broader range of replication rate and cross-immunity values.

3.4 Effects of transmission bottlenecks and within-host fitness

In order to understand how measures of within-host viral fitness translated to between-host fitness through transmission bottlenecks, we simulated transmission along a linear chain of hosts with pre-determined initial immunity. Each host-to-host transmission event occurred once. Hosts in each transmission chain were either: all naive, all experienced or alternating between naive and experienced. In a homogenous chains of naive hosts, DE was driven to extinction relatively rapidly by both methods of transmission due to competition from wildtype (Supplementary Fig. 2A and C, compare to single infections B and D). In experienced hosts, DE could persist for several transmissions (although was eventually outcompeted by W, result not shown) when within-host fitness depended on peak viral load (Supplementary Fig. 2E) whereas the mutant could outcompete W when its relatively long infectious period was accounted for (Supplementary Fig. 2G). The outcome was similar when the host chain alternated between naive and experienced hosts although it took longer for DE to outcompete W by mean- load-based transmission, and DE was lost faster by peak-load- based transmission. If the mutant has a replication advantage, it is always predicted to outcompete W regardless of which measure of within-host fitness is used to determine transmissibility (although AE wins fastest in experienced hosts as expected). These results predict that a mutant with a replication advantage would always be selected over one with a

replication deficiency and that a mutant with a replication deficiency would need a longer infectious period to persist.

3.5 Infection of hosts with immune memory

In order to understand how the interdependence of within- host dynamics and immune memory impacted selection of a replication-deficient mutant, we simulated transmission by re-infecting the same host using the immunity levels that had arisen in the immediately previous infection (Fig. 3). When DE was introduced alone (Fig. 3B, F, D, H), it did not survive to transmission of secondary infection since the DE-primary effector response was high enough in the initial host. On the other hand, when DE arose during an infection of W (Fig. 3A, E, C, G), only very low levels of E-primary effectors were stimulated while high levels of W-primary effectors arose. This OAS effect acted to suppress W to extinction during the first reinfection which alleviated competitive suppression. It also allowed DE to persist through multiple re-infections since the original infection had stimulated such a strong W-primary response, which amplified the action of OAS during each re-infection. The results were similar regardless of transmission method or immune status in the initial host. However, we found that the magnitude of waning of immunity between infections affected persistence of DE differently depending on host immunity status in the initial infection. If immunity was allowed to wane to a lower amount between infections (W = 0.75), then DE could persist in single infections of experienced hosts (Fig. 3D and H) since the prior experience with W caused some degree of OAS. If immunity waned less between infections (W = 0.85), then DE only persisted following co-infection of naive hosts since this is when OAS is strongest. In summary, the results show that: (1) co-infections alter the probability of immune escape through their effects on the immunity structure in the host population, and (2) when re-infections are considered, an immune-escape mutant with a replication deficiency has a higher probability of success than would be expected from its dynamics in a host population where only primary infections occur (i.e., Supplementary Fig. 2).

3.6 Effect of mutant replication in initial host and heterogeneity in host-chain immunity

To investigate how replication rate would affect mutant selection in a more complex population, we compared transmission success of the mutant through hosts that had random levels of experience with W to those which had immune memory from prior infections and combinations of these types of hosts. Fig. 4 shows that in a population with random levels of experience to W, when immunity was higher than average in the first host, a replication-deficient mutant could succeed for several transmissions, whereas it went quickly extinct when starting in a naïve host (Fig. 4B). Oppositely, if DE was transmitted first to a host with a random amount of experience to W and then re-infected the initial host, it is predicted to survive only if it initially arose in a naive host (Fig. 4J). This illustrates that the structure of immunity in the host transmission chain and the immune status wherein the mutant arises critically determine mutant persistence. (Note that the differences in immunity levels in previously infected hosts are more sensitive to initial immunity in prior infections rather than viral dose, Supplementary Fig. 4). When the initial host was experienced with W, strong OAS resulted, but total immunity was also very high. The subsequent contact host which had a random level of W-primary effectors allowed W to persist to the third infection

(re-infection of the initial host). Co-infection of this initial host (which had very high levels of W-primary effectors) then facilitated an even stronger immune response which drove both W and DE to extinction. Persistence of DE was allowed during serial re-infections (Figs. 3 and 4F) due to exclusion of W upon the first re-infection.

On the other hand, when the mutant with a replication advantage was transmitted to a host with a random level of W-primary effectors before re-infection of the initial host, it went extinct upon re-infection regardless of whether it arose in a naive or experienced host since it was able to stimulate a strong E-primary immune response in the initial infection (Fig. 4L). Although, AE could survive during serial re-infections when it initially arose in a host with strong W-primary immunity since competition and immune-mediated interference from W was alleviated immediately and the effect of OAS was strong (Figs. 4H and 1I). However, unlike the replication-deficient mutant, AE could not persist through serial re-infections if it initially arose in a naive host since strong E-primary immunity existed (Figs. 4H and 1F). These differences in predicted persistence of AE and DE were qualitatively similar for both methods of transmission (Fig. 4 and Supplementary Fig. 3). Together the results emphasize that the optimal replication rate of an immune-escape mutant depends on the initial immunity of the host wherein the mutant arises, the amount and heterogeneity of W-primary effectors in the host population and the frequency of re-infection. Specifically, in host contact networks where re-infections are frequent and the host population is relatively naive, an immune-escape mutant with a low replication rate relative to wildtype (and that cross- reacts with W-primary effectors) would be favored.

3.7 Effects of multiple transmissions per host

In order to examine how immune selection in re-infections impacted the evaders when multiple transmissions per host were allowed, we simulated transmission of the two evaders in a randomly mixing host population. In contrast to predictions from the linear chains in Fig. 4, when re-infections were not allowed, the replication-deficient evader was able to persist as long as wildtype due to its longer infectious period in co-infections (Fig. 5A). The replication-advantage evader, on the other hand, showed similar dynamics as in the linear chains (rapidly outcompeted wildtype, causing a larger number of infections; Fig. 5B). When re-infections were allowed, DE caused more total infections than AE and persisted for longer (Fig. 5C–D), which was qualitatively similar to predictions from the linear chains (Fig. 4F–H, solid lines). The epidemic time series of mean immunity in recovered hosts (Fig. 5E–H) showed that OAS was stronger for epidemics initiated with DE relative to AE. Changes in immunity levels were due to changes in the frequency of different types of infections (single versus co) and their effects on infectious period (note that single infections have shorter infectious periods and thus hosts that were infected by a single strain initially will appear earlier in the immunity time series and will be available for secondary infection earlier). The time series profile of types of hosts that were infected by E (Fig. 5I–J) illustrates that the superior transmission of DE relative to AE was due to re-infection of hosts that recover from single infections with W, which appeared earlier in the epidemic time series due to the shorter infectious period for the W single infections. AE did not gain this advantage since it prevented the spread of W initially and was limited to re-infection of

hosts that it had already infected (which had an elevated level of E- specific immunity due to high E-replication rate).

4. Discussion

Viral immune escape is particularly challenging to predict since selection by immunity is directly modified by viral epidemiological dynamics and acts across multiple scales (within hosts and at the host-population scale) (Grenfell et al., 2004). In order to examine how the interdependence of viral dynamics and immune pressure impact immune escape, we used a nested model of within- and between-host viral and immunity dynamics. Within-host dynamics depended on viral competition, cross-immunity and production rates of immune effectors. These infection dynamics determined transmissibility of strains and host immunity at recovery which then influenced within-host dynamics and transmissibility in subsequent infections. We showed that immune-escape risk depends strongly on the viral dynamics in previous infections, and that replication- deficient mutants can be favored over those with higher replication rates since they cause stronger original antigenic sin. This advantage from strong OAS would be expected to occur in situations where viral persistence depends on re-infections of previously exposed hosts, for example, when host contact is highly structured and limited to local interactions.

4.1 Within-host interactions create an advantage for mutants with a replication deficiency

When considering the effects of strain interactions within hosts on immunity in recovered hosts, we found that OAS caused selection of the replication-deficient immune-escape mutant and near extinction of wildtype during secondary infection regardless of immune status in the primary infection. Competitive suppression in the primary infection acted to amplify the magnitude of OAS since it prevented DE from being amplified to a degree that stimulated a strong E-primary response. The high W-primary immune response effectively removed that host from the pool that was susceptible to W, leaving it almost completely naive to E. Thus, at the population level, the combined effects of competition and OAS can provide a higher than expected advantage for an immune-escape mutant with a replication deficiency. This result emerges from explicit consideration of within-host co-infection dynamics on host-population immunity. A previous nested model that examined selection on viral replication rates (and included > two strains by mutation) similarly found that reduced replication can be favored, albeit through a different mechanism (Alizon and van Baalen, 2008; Luciani and Alizon, 2009). They showed that when longer infectious periods optimize between-host fitness, reduced replication rates can evolve since they allow for better immune evasion and longer infectiousness which allows for increased production of mutants. Together the results emphasize the importance of linking immune-selection pressures across the within-host and host-population scales in order to predict the risk of immune escape. They also provide alternative explanations for the frequent occurrence of immune-escape mutants with replication deficiencies; that is, they can be selectively advantageous over mutants that lack a replication deficiency rather than occurring strictly due to genetic constraints.

4.2 Host-contact structure selects immune-escape phenotypes

We found that the initial immunity of the host wherein the mutant arose and the immunity structure of the other hosts in transmission chain, could critically determine immune escape risk and select replication rates of immune-escape mutants. Replication-deficient mutants would be most successful when re-infections are frequent, whereas mutants with high replication rates would be most successful when much of the host population has experience with W and re-infections are uncommon. This suggests that in highly structured populations, where individuals have few contacts and global movement is much less frequent than local interactions, an immune-escape mutant with a replication deficiency could be selectively advantageous over one with a high replication rate since it would outcompete other strains in re-infections. Our observation that the optimal viral phenotype for immune escape depends strongly on contact structure between hosts in the first few infections is consistent with previous work showing that host-network structure and contact rates affect evolution of viral replication rates (Boots and Mealor, 2007; Lange and Ferguson, 2009; Read and Keeling, 2006). However, in contrast to our finding, previous work found that viral replication rate evolved to be higher when host contact is limited locally relative to when global interactions are allowed (Read and Keeling, 2006). This previous result emerged due to a trade-off between strong competition in locally connected networks (where fast replication is optimal) and persistence in globally connected networks (where long infectious periods are optimal). Moreover, the previous study did not consider the role of re-infected hosts and thus did not include effects from infection-induced immunity and OAS, which would explain the discrepancy in results. Future studies will need to address how within-host dynamics modify transmissibility (rather than exclude transmission completely) along edges of the host-population contact network in order to predict which viral traits present the highest risk of immune escape in structured host populations.

4.3 Heterogeneity in host-population immunity

Both the linear chains and the randomly mixing population models showed that reduced viral replication rates can be selectively advantageous when heterogeneities in host-population immunity arise during the epidemic time course. The advantage arises from a combination of transmission dynamics and OAS. The slower replicating evader is excluded from some transmissions early during the epidemic time course, allowing for increased levels of W infections which have shorter infectious periods (due to the lack of competition and high replication rate). Hosts that recover from the W single infections are the first to become available for re-infection and have a high level of W-primary immunity. This enables strong OAS in re-infections such that multiple re-infections with E can occur while re- infections with W are excluded. However, AE does not gain this type of advantage since it rapidly excludes W-infections. Other nested models have predicted that lower replication rates can be favorable under elevated host-population heterogeneity due to sources such as random differences in within-host immunity parameters (Ganusov et al., 2002), imperfect vaccines (Ganusov and Antia, 2006a) or vaccination coverage (Andre and Gandon, 2006), and on-going infections (Alizon and van Baalen, 2008a). However, the last study was the only study besides ours that considered virus-induced heterogeneity that arises during the epidemic time course, and their result (as well as predictions from the other studies) depended on the assumption that higher parasite loads lead to higher virulence. We found

that host- population heterogeneity from virus-induced epidemic patterns could select for lower viral replication rates even when replication rate is not linked to virulence.

4.4 Simplifying assumptions

In order to maintain transparency of the effects of multiple within-host interactions on host-population immunity, some simplifications of within-host biology were necessary. We assumed that there was only one type of immune-system component (effectors acting directly on virus) and that levels of effector-producing cells were proportional to levels of effectors produced during the previous infection. This latter assumption is based on data showing that levels of immune cell recruitment depend on viral load and determine levels of mature strain-specific memory cells (Hikono et al., 2006). However, our former assumption neglects the well-known fact that innate immunity is often the first line of defense against viruses that cause acute infections; because of this simplification, our model does not aim to predict the absolute timing of peak load and viral clearance. Although our within-host model is simple, it qualitatively captures the effects of viral competition and OAS (Kim et al., 2009; Pepin and Hanley, 2008), and serves to emphasize that within-host dynamics are important for predicting rates of immune escape and assessing vaccine efficacy. More complex, realistic within-host dynamical models that include multiple immune system components have recently been developed for interpreting the timing of events within hosts more accurately (Antia et al., 2005; Baccam et al., 2006; Hancioglu et al., 2007; Lee et al., 2009). However, more empirical data (i.e., strain interactions within hosts, their effects on immune response and how immune history impacts the within-host dynamics in previous infections) is needed before these parameter-rich models can be applied to study selection of immune-escape mutants across the within-host and population scales.

In order to understand the interdependence of viral phenotype on immune-selection strength, we considered only two strains and only the evolutionary dynamics due to selection. Thus, we neglected effects from the time and rate at which immune-escape mutations occur, as well as effects of selection in genetically diverse populations, both of which are known to be important determinants of fixation probability of advantageous mutations (Gerrish and Lenski, 1998; Miralles et al., 1999; Pepin and Wichman, 2008; Wahl and Gerrish, 2001; Wilke, 2004). These evolutionary processes should be accounted for in order to predict sustainability time and efficacy of viral disease interventions. Furthermore, a recent model has shown that selection for reduced replication rate can lead higher production of escape mutants since decreased replication is correlated with longer replication times (Luciani and Alizon, 2009). An understanding of how these effects of mutation and selection within-hosts impact the structure of population immunity is an important future direction for both theoretical and empirical research on the evolutionary dynamics of immune escape.

4.5 Perspectives for minimizing the risk of viral immune-escape

Our model shows that effects of within-host interactions on host-population immunity determine the risk of immune escape. Furthermore, the immune-escape phenotype with the highest transmission success depends critically on re-infection dynamics and host-contact network: a replication-deficient mutant can be favored over one with high replication when re-infections occur early after initial emergence. These are important considerations for the

design and implementation of disease control methods that impact viral replication rates (i.e., drugs, live-attenuated vaccines) and underscores the importance of considering interactions between strains and multiple phenotypic traits of viruses when developing interventions. Since mutations affecting anti- body recognition are often pleiotropic on replication rate (Berkhoff et al., 2007; Novella et al., 2005; Rimmelzwaan et al., 2005; Rudneva et al., 2005), it is important to consider how pleiotropic fitness landscapes shape immune-escape dynamics. In order to develop disease intervention methods that minimize transmission of immune-escape mutants, future studies should aim to explain the changing profile of host immunity through the linkage of within-host interactions and host-contact structure, using realistic viral genotype-fitness maps.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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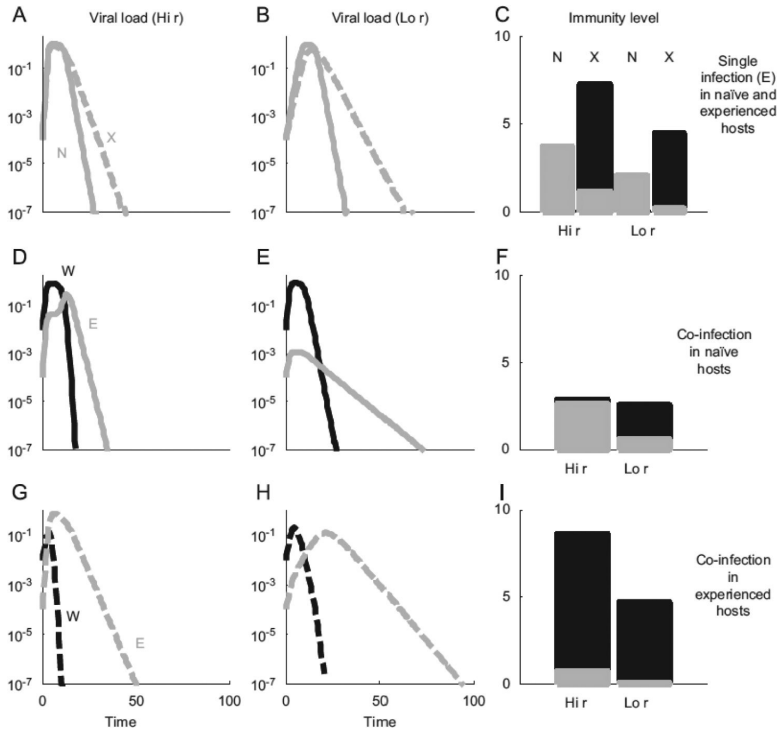

Fig. 1.

Within-host dynamics. Viral load ($\log_{10}$) over time (columns 1 and 2) and levels of specific immune effectors at recovery (column 3) are shown for the wildtype (W, black) and mutant (E, gray). (A) Single infections of E in naive hosts (solid line; $I_{0W} = I_{0E} = 10^{-3}$) and hosts with prior experience with W (dashed line; $I_{0W} = 1$, $I_{0E} = 10^{-3}$) under high replication rate ($\rho_E = 2.7$). (B) Same as (A) but under low replication rate ($\rho_E = 1.1$). (C) Immunity at recovery for infections in plots A and B (bar labels are: N: naive hosts, E: experienced). (D) Co-infections in naive hosts when E has a replication advantage ($\rho_E = 2.7$, $\rho_W = 2$). (E) Co-infections in naive hosts when E has a replication deficiency ($\rho_E = 1.1$, $\rho_W = 2$). (F) Immunity levels for D and E. Plot G–H are similar except hosts are experienced. Initial viral doses were asymmetric: $V_{0W} = 10^{-2}$ and $V_{0E} = 10^{-4}$. Cross-immunity: $\varepsilon_W = 0.8$, $\varepsilon_E/\varepsilon_W = 0.35$.

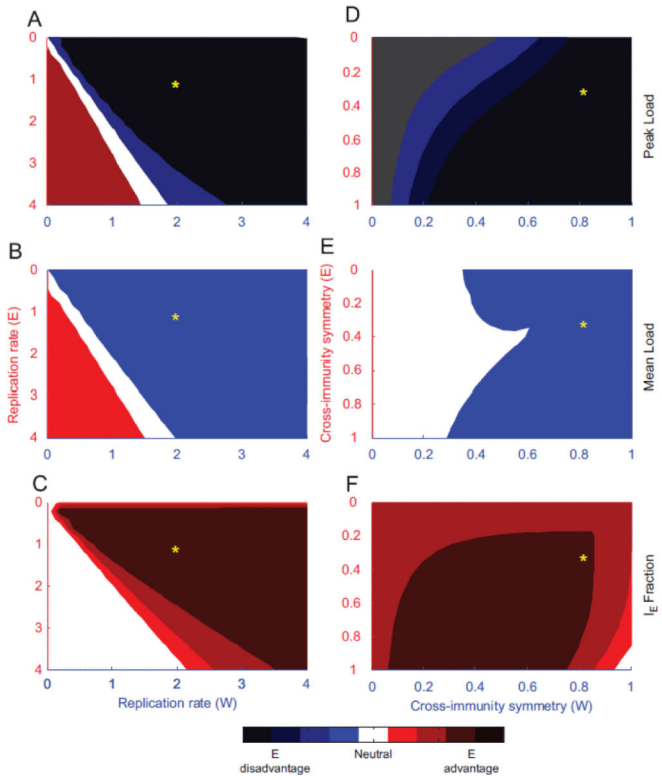

Fig. 2.

Interaction of replication rate and cross-immunity traits. Three measures of within-host viral advantage (indicated at right) as a function of replication rates (A–C) and cross-immunity (D–F). Peak load (A and D) is the difference of E and W peak loads; mean load (B and E) is the difference of E and W average loads during entire infectious period; IE immunity (C and F) is the difference between co-infections and single infections in the fraction of immunity that is specific against E. Gray indicates an advantage for E in either viral abundance or OAS (under-stimulation of E-specific immunity). Fixed parameters: $\varepsilon_W = 0.8$, $\varepsilon_E/\varepsilon_W = 0.35$ (A–C); $\rho_W = 2$, $\rho_E = 1.1$ (D–F), $V_{0W} = 10^{-2}$, $V_{0E} = 10^{-4}$; $I_{0W} = I_{0E} = 10^{-3}$ (naive host). Stars indicate the parameter values used in all other analyses.

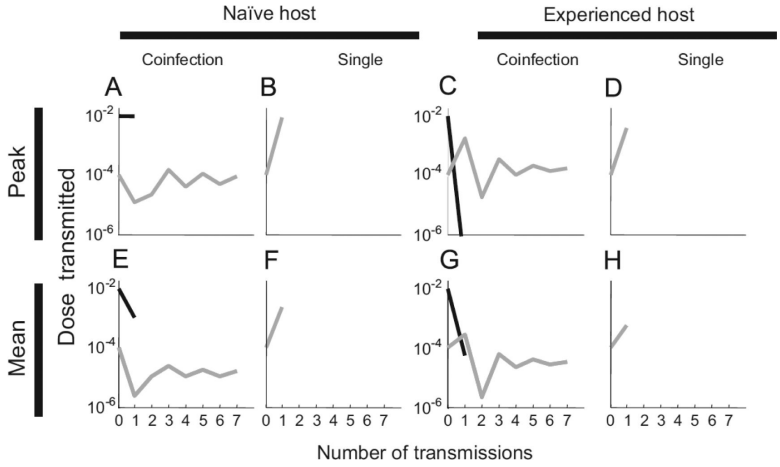

Fig. 3.

Effects of co-infection dynamics in the initial host on re-infection of the same host. Trajectory of viral doses available for subsequent re-infections of the same host (W, black; E, gray). Hosts maintained the same relative amount of wildtype- and mutant-specific immunity that arose in the prior infection. Persistence of the mutant is compared for hosts that were initially co-infected (A, C, E, and G) versus those that were infected only by the mutant (B, D, F, and H). *X-axes* are the number of transmissions (i.e., 0 indicates the doses used to initialize the first infection whereas 1 indicates the doses transmitted at the end of the first infection). Doses were determined at peak viral load (A–D, Method 1 in Section 2) or by average viral load (E–H, Method 2 in Section 2). The initial host was either naive ($I_{0W} = I_{0E} = 10^{-3}$; columns 1 and 2), or had prior experience with W ($I_{0W} = 1$, $I_{0E} = 10^{-3}$; columns 3 and 4). Fixed parameters: $\rho_W = 2$, $\rho_E = 1.1$; $\varepsilon_W = 0.8$, $\varepsilon_E/\varepsilon_W = 0.35$; $V_{0W} = 10^{-2}$, $V_{0E} = 10^{-4}$; bottleneck at transmission (B) = 10^{-2}; minimum threshold on infectious load (M) = 10^{-7}; waned immunity (W; proportion of total immunity retained) = 0.8.

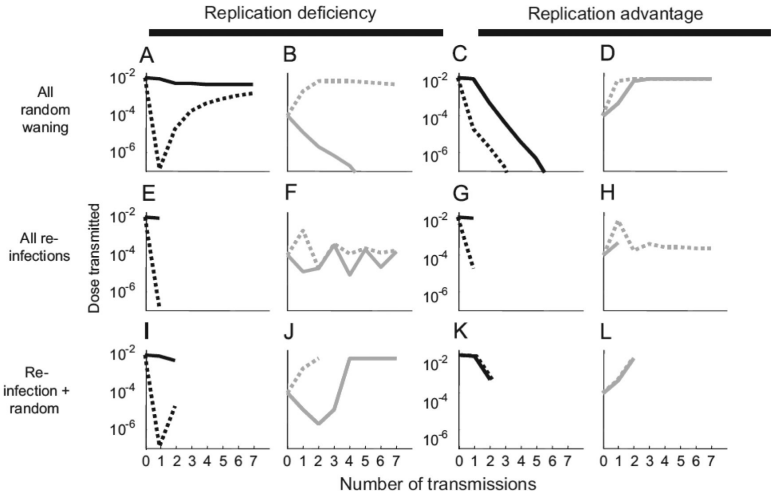

Fig. 4.

Effects of replication rate and host-population heterogeneity. Trajectory of viral doses available for subsequent infections (W, black; E, gray). (A–D) Hosts with a random amount of W-specific immunity (I_{0W} ~ U[0.001, 1]). (E–H) Re-infection of the same host as in Fig. 3 (W = 0.82). (I–L) One infection with a random amount of waned W-specific immunity (as in A–D) followed by re-infection of the first host in the chain and then again followed by random immunity levels in host infections 4–7. *X-axes* are the number of transmissions (i.e., 0 indicates the doses used to initialize the first infection whereas 1 indicates the doses transmitted at the end of the first infection). Replication rates: ρ_W = 2, ρ_E = 1.1 (columns 1 and 2), and ρ_W = 2, ρ_E = 2.7 (columns 3 and 4). Doses were determined at peak viral load (A–D, Method 1 in Section 2). The initial host was either naive ($I_{0W} = I_{0E} = 10^{-3}$; solid lines), or had prior experience with W (I_{0W} = 1.5, $I_{0E} = 10^{-3}$; dotted lines). Fixed parameters: ε_W = 0.8, $\varepsilon_E/\varepsilon_W$ = 0.35; $V_{0W} = 10^{-2}$, $V_{0E} = 10^{-4}$; bottleneck at transmission (B) = 10^{-2}; minimum threshold on infectious load (M) = 10^{-7}.

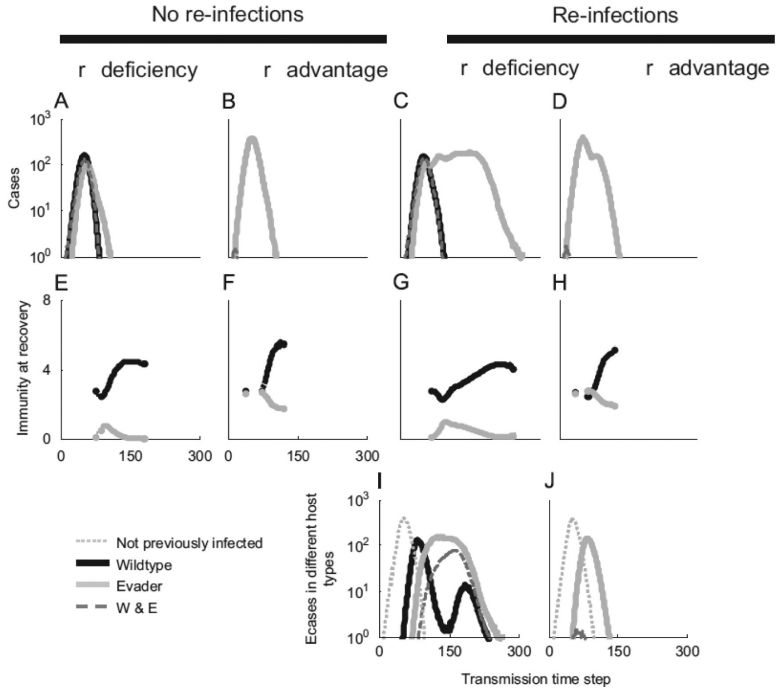

Fig. 5.

Effects of replication rate and host-population heterogeneity under multiple transmissions per infection. Epidemic dynamics in a randomly mixing population of 10,000 hosts. Outputs are means for 100 simulation runs under each of four conditions: low replication evader without (A, E) or with (C, G, I) re-infections and high replication mutant without (B, F) and with (D, H, J) re-infections. Each run was initiated with a single co-infected host (V_{0W} = 10^{-2}, V_{0E} = 10^{-4}, I_{0W} = I_{0E} = 10^{-3}; equal to initial conditions in Fig. 4 solid lines). The initial immunity in uninfected hosts was heterogeneous (as in Fig. 4A–D). (A–D) Mean number of new wildtype- (solid, black), evader- (solid, gray), and co- (dashed, dark gray) infected hosts per transmission time step. (E–H) Mean immunity in hosts that recovered at each time step. (I–J) Number of different host types that are infected by the evader (either alone or in co-infection) at each time step. All other parameters were as in Fig. 4.