Immune Lag Is a Major Cost of Prokaryotic Adaptive Immunity During Viral Outbreaks

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Abstract

CRISPR-Cas adaptive immune systems enable bacteria and archaea to efficiently respond to viral pathogens by creating a genomic record of previous encounters. These systems are broadly distributed across prokaryotic taxa, yet are surprisingly absent in a majority of organisms, suggesting that the benefits of adaptive immunity frequently do not outweigh the costs. Here, combining experiments and models**,** we show that a delayed immune response which allows viruses to transiently redirect cellular resources to reproduction, which we call "immune lag", is extremely costly during viral outbreaks, even to completely immune hosts. Critically, the costs of lag are only revealed by examining the non-equilibrium dynamics of a host-virus system occurring immediately after viral challenge. Lag is a basic parameter of microbial defense, relevant to all intracellular, post-infection antiviral defense systems, that has to-date been largely ignored by theoretical and experimental treatments of host-phage systems.

Introduction

CRISPR-Cas immune systems are the only known form of adaptive immunity found in prokaryotic organisms [1, 2]. These antiviral defense systems enable bacteria and archaea to incorporate short stretches of viral genetic material into specific loci on the host genome (the CRISPR "array") as individual immune memories, called "spacers" [2]. Spacers are later transcribed and processed into crRNA sequences that guide CRISPR-associated (Cas) proteins to cleave viral nucleic acids [3, 4, 5]. Thus, a genomic record of past infections is used to prevent future infection (see [6] for a recent review of the mechanisms of CRISPR-Cas immunity).

CRISPR-Cas systems are widely but sparsely distributed across the tree of life [7, 8, 9]. Their broad distribution among taxa is likely attributable to the fact that these systems are highly effective

at clearing viral infections (*e.g.,* [2]), extremely adaptable in a constantly shifting co-evolutionary arms race [10, 11], and, similarly to other defense systems [12], frequently horizontally transferred [13, 12, 14, 15] (for reviews of various aspects of CRISPR biology see refs. [16, 17, 9, 18]). Yet, a majority of prokaroytes lack CRISPR-Cas immune systems [19], even as CRISPR-Cas can usually be found in a closely-related relative. To solve this apparent paradox various authors have proposed a number of costs and limitations of CRISPR-Cas immunity that may drive selection against this system in favor of alternative defense strategies (of which there are many [20]). These "cons of CRISPR" potentially include autoimmunity [21], the inhibition of beneficial horizontal gene transfer [22, 23], the inhibition of other cellular processes by the *cas* genes (specifically DNA repair; [24, 25]), incompatibility with lysogenic phage [26], and the possibility that CRISPR-Cas may be unable to keep up with extremely diverse pathogenic environments [27, 28]. Nevertheless, experiments show that CRISPR-Cas systems can be essentially cost-free in phage-free culture conditions [29, 30, 31].

In contrast to results indicating that CRISPR-Cas defense generates little-to-no constitutive costs for the host in the absence of phage – at least in lab-reared *Pseudomonas aeruginosa* – a severe inducible cost of CRISPR-Cas immunity upon phage infection has been observed [29]. The source of the inducible cost of CRISPR-Cas immunity was, until recently, mysterious. Importantly, while CRISPR-immune cells were observed to have reduced fitness when exposed to phage in competition experiments, subsequent efficiency of plating experiments showed that CRISPR-immune cells did not experience phage-induced mortality [29], indicating that phage inhibit the growth of immune cells but do not kill them. Chabas et al. [32] suggested that the inducible cost is the result of transient expression of phage genes in the cell before CRISPR-Cas is able to clear an infection (figure 1). Recently Meaden et al. [31] have provided evidence confirming this lag hypothesis in experiments with *Pseudomonas aeruginosa* strain PA14 and its phage DMS3*vir*, demonstrating that phage gene expression is responsible for a reduction in the fitness of CRISPR-immune host. Specifically, Meaden at al. [31] showed that a phage protease was transiently expressed in CRISPR-immune cells before infection could be cleared, and that expression of this gene was detrimental to host fitness. When a virus infects a cell, viral genes will be expressed, often at very high levels. At the same time the host cell's expression patterns may be "reprogrammed" by the infecting virus (creating a "virocell"; [33, 34, 35]). Intracellular DNA- or RNA-degrading defenses may take some time to find and degrade invading genetic material in the cell, and during that time transcription in an infected cell may be transiently altered [34, 35], potentially halting host growth and re-purposing cellular resources. This phenomenon, which we call "immune lag", was observed by Meaden et al. [31] in CRISPR-immune cells, so that even when cells are able to effectively clear infections and prevent lysis they still pay a heavy growth cost associated with infection (figure 1). Could immune lag be a major cost of adaptive immunity, leading the host to sometimes favor alternative immune strategies?

Upon closer examination, the impact of immune lag on natural systems is less clear. Experiments that demonstrate the inducible cost of CRISPR-Cas immunity require the host to be exposed to extraordinarily high viral titres (at least 10^8-10^{10} PFU/mL in our own experiments, described below) to see any effect. For lag to have any population-level impact on an immune host population, a substantial portion of the immune population must be exposed to phages. Thus, in the case of an already-immunized host population, lag is probably irrelevant because viruses have no way to reach sufficiently high titres to suppress the immune host.

Yet, in natural systems, host populations are rarely completely immune to their viral pathogens. CRISPR spacers can be lost [36, 37, 22, 38, 39, 15], and viral escape mutants with point mutations in protospacer regions frequently emerge [40, 41], both leading CRISPR-Cas to be a somewhat

transient form of immunity [31]. In natural communities, entirely new species of virus, to which the host lacks preexisting immunity, may migrate into the system via dispersal [42]. Thus, to fully characterize the role of immune lag in natural systems, we must assess its impact on non-equilibrium systems with viral coevolution or migration. We combined experiments and mathematical models to investigate how lag transiently alters the costs of CRISPR-Cas during a viral outbreak, and found that when viruses invade a primarily susceptible host population with a small sub-population of CRISPR-immune host even the CRISPR-immune cells face a large virus-induced reduction in fitness. Importantly, the costs of lag are only revealed by examining the dynamics of the system that occur immediately after viral challenge.

Models

Model Framework

To model CRISPR-Cas immunity in a simple host-phage system, we built on classical host-phage chemostat (or "virostat", see discussion below) models [43, 44, 45], where resources (R) are modeled explicitly as being supplied by some constant reservoir (r_0), and there is constant flow (w) of resources into the system. At the same time, the contents of the system (cells, viruses, resources) are removed at the same rate (w) in order to maintain a constant volume. For a detailed discussion of this class of models see Weitz 2016 [45]. Our model consists of a system of ordinary differential equations with equations for resources, host, and virus populations:

$$
\begin{aligned}
\overbrace{\dot{R}}^{\text{Resources}} &= \overbrace{w(r_0 - R)}^{\text{Flow}} - \overbrace{\frac{evR}{z+R}(S+C)}^{\text{Resource Uptake by Cells}} \\
\overbrace{\dot{S}}^{\text{Susceptible Host}} &= \left(\overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Flow}} \right) S \\
\overbrace{\dot{C}}^{\text{Immune Host}} &= \left(\overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{w}^{\text{Flow}} \right) C + \overbrace{\mu \delta V S}^{\text{Immunization}} \\
\overbrace{\dot{I}}^{\text{Infected Host}} &= \overbrace{(1-\mu)\delta V S}^{\text{Infection}} - \overbrace{\gamma I}^{\text{Lysis}} - \overbrace{wI}^{\text{Flow}} \\
\overbrace{\dot{V}}^{\text{Viruses}} &= \overbrace{w(v_0 - V)}^{\text{Flow}} + \overbrace{\beta \gamma I}^{\text{Lysis}} - \overbrace{\delta (S+C) V}^{\text{Adsorption}} .
\end{aligned}
\tag{1}
$$

Specifically, we equipped our host population with a CRISPR-Cas system, so that there is a population of naive, undefended-but-CRISPR-encoding host (S) that may become infected (I) by viruses (V) and may also undergo immunization at rate μ to become defended (*i.e.,* spacer-possessing) host (C). This formulation is similar to other minimal models of CRISPR-Cas immunity (*e.g.,* [46, 47, 48, 39, 49]). For some analyses we also included a virus-resistant surface mutant (SM)

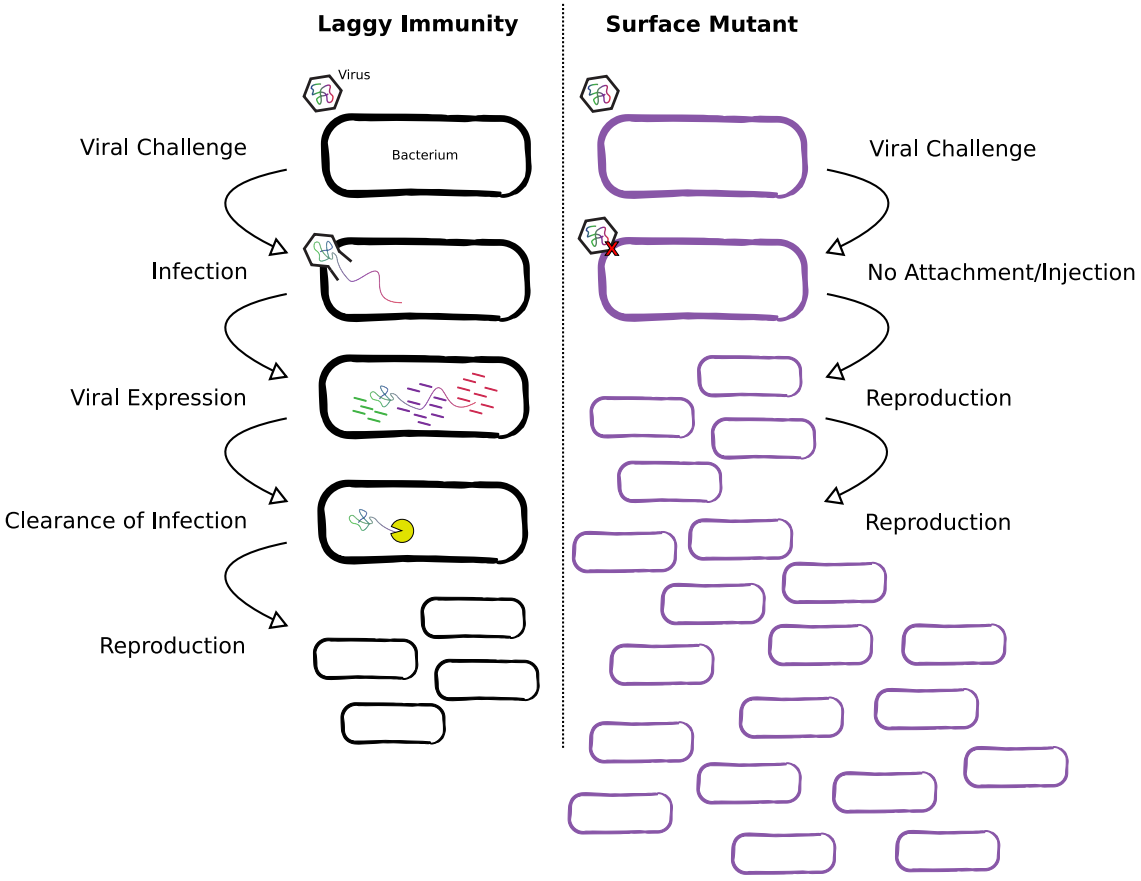

Figure 1: **During competition, immune lag can lead infected cells to face significant delays in reproduction while cellular resources are temporarily diverted to viral production before the infection can be cleared via cleavage of viral nucleic acids (left).** In contrast, an SM strategy does not allow viral genetic material to enter the cell in the first place, preventing any lag (right).

strain in the model (M):

$$\overbrace{\dot{M}}^{\text{SM Host}} = \left(\frac{\overbrace{(1-\kappa)vR}^{\text{Growth}}}{z+R} - \overbrace{w}^{\text{Flow}} \right) M \tag{2}$$

with growth cost κ associated with it's surface mutation, and appropriate changes to our equation for resource dynamics.

Our model of CRISPR-Cas immunity is intentionally simple in that it neglects (i) details of the spacer acquisition process, (ii) autoimmunity, (iii) spacer diversity, and (iv) viral coevolution. CRISPR-immune strains are modeled as a single, homogeneous pool of immune host (C) and viruses are not able to coevolve to overcome CRISPR-Cas immunity. Nevertheless, this model is a suitable scaffold on which to build a more complex model of immune lag. We provide detailed explorations of the spacer acquisition process in S1-S2 Text and S1-S3 Figs (including acquisition from collapsed replication forks [50, 51], acquisition from defective phages [52], and primed acquisition [10, 11]), and show that the details of spacer acquisition are largely irrelevant for assessing the fitness cost of lag in CRISPR-immune host. Furthermore, a careful analysis of autoimmunity, with rates estimated based on a realistic model of self versus non-self recognition (S1 Text), predicted that there should be essentially no impact of autoimmunity on the hosts' fitness (S1 Figure), which is consistent with experimental efforts that have not detected any constitutive cost of CRISPR-Cas immunity [29, 30, 31]. We address spacer diversity and viral coevolution in our simulations of repeated viral outbreaks (see Results, Methods).

Finally, observe that in a small departure from the classical chemostat model we allow constant immigration of viruses into the system from some environmental pool (v_0). This is an entirely experimentally tractable modification (*e.g.,* by adding set concentrations of virus to the resource reservoir), and better represents natural systems which are not closed and where hosts likely face constant challenges in the form of newly-arriving viruses. Note that this basic model only considers a single viral genotype, so that immune hosts will also be immune to immigrating viruses (though see outbreak simulations discussed later for simulations in which this is not the case). For traditional continuous culture without viral inflow simply let $v_0 = 0$.

Immune Lag

Meaden et al. [31] provide strong evidence that the inducible cost of CRISPR-Cas is associated with transient expression of viral genes and possible virus-induced reprogramming of cellular transcriptional networks. Because CRISPR-Cas immunity does not remove viral genetic material from the cell instantaneously, Meaden et al.'s [31] results suggest that even immune hosts face a temporary growth setback during infection while viruses transiently reprogram the cell (figure 1). Virus-resistant surface mutants do not experience this growth setback, as viruses are unable to adsorb to the cell in the first place. Consider the simple model above. We add an equation for transiently infected but immune host (L):

$$\overbrace{\dot{L}}^{\text{Lagged}} = \overbrace{\delta CV}^{\text{Infection}} - \overbrace{\phi L}^{\text{Clearance}} - \overbrace{wL}^{\text{Flow}} \tag{3}$$

which are able to clear an infection at rate ϕ via cleavage of viral nucleotides, and modified the equation for immune host accordingly:

$$\dot{C} = \left(\overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Flow}} \right) C + \overbrace{\mu \delta V S}^{\text{Immunization}} + \overbrace{\phi L}^{\text{Clearance}} . \quad (4)$$

We also found that lag can be modeled in an even simpler four-parameter system with qualitatively similar results (S4 Text and S4 Fig). Thus, for completeness and comparison with experimental results, we present a parameter-rich model, but our results can be replicated with minimal models of host-phage interactions.

Upregulation of the CRISPR Locus

The *cas* genes and CRISPR arrays of many hosts are transcriptionally upregulated in response to infection [53, 54], or in situations where there is a high risk of infection (*e.g.,* in a biofilm; [55, 56, 57, 58, 59]). The specific regulatory cues used by the CRISPR locus are diverse [54], and new methods are being developed to probe them [60]. Consider the case where the CRISPR locus is specifically upregulated in response to infection. The initial time-to-clearance of infection is unaffected by upregulation, but for some time after this first infection the host will be on "high alert", producing many Cas proteins and crRNAs. We consider the case where this overproduction of CRISPR-Cas defense complexes allows the host to degrade viral genetic material before it can be expressed, thus avoiding any immune lag. We implemented this scenario by letting recently immunized and lagged cells pass into a "fast" immunity (C_F) state where the CRISPR-Cas system does not experience immune lag because the *cas* targeting genes are upregulated:

$$\dot{C}_F = \left(\overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{w}^{\text{Flow}} \right) C_F + \overbrace{\mu \delta V S}^{\text{Immunization}} + \overbrace{\phi L}^{\text{Clearance}} - \overbrace{\zeta C_F \left(\frac{C_F}{C_F + \delta V} \right)}^{\text{Downregulation}} \quad (5)$$

and modified the equation for immune host accordingly:

$$\dot{C} = \left(\overbrace{\frac{vR}{z+R}}^{\text{Growth}} - \overbrace{\delta V}^{\text{Infection}} - \overbrace{w}^{\text{Flow}} \right) C + \overbrace{\zeta C_F \left(\frac{C_F}{C_F + \delta V} \right)}^{\text{Downregulation}} \quad (6)$$

Note that we do not include any cost of increased transcription/translation in this model, as we have no empirical estimate or intuition for the scale of this cost, though one almost certainly exists (since in the absence of a cost the expression of CRISPR-Cas would be expected to be constitutively high). Also, observe that we modeled an upregulation of the *cas* targeting genes, which will reduce or eliminate immune lag (in our case eliminate), rather than the *cas* acquisition machinery, which would possibly increase autoimmunity (though both may be upregulated during infection since the *cas* genes are often transcribed as an operon). Finally, the return to a downregulated state will be prevented if there are still many viruses in the environment infecting our "fast" immune cells, hence our inclusion of a nonlinear downregulation rate that will approach zero at high multiplicity of infection (MOI).

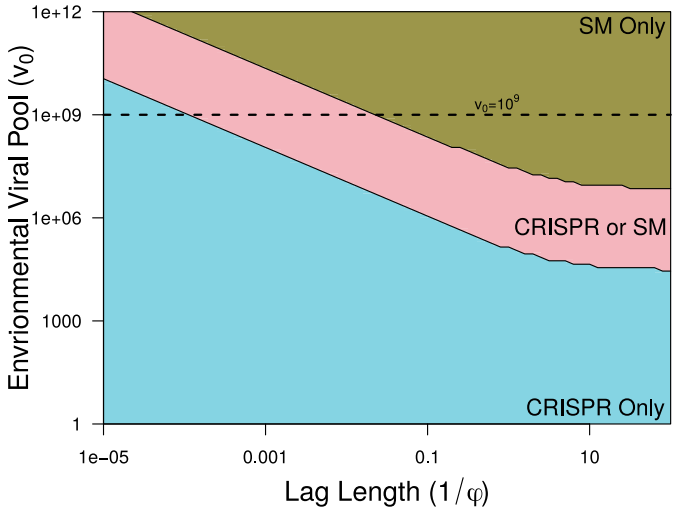

Figure 2: **Model equilibria show a transition from complete reliance on CRISPR-Cas immunity to complete reliance on surface modifications by the host at very high viral titres.** No parameter conditions leading to stable coexistence of the two host strains were observed. Blue region denotes region of parameter space in which the CRISPR-only equilibrium was the only stable equilibrium and brown denotes the region in which the SM-only equilibrium was the only stable equilibrium. Pink denotes the region in which both CRISPR-only and SM-only equilibria were stable, indicating that the final state of the system depends on initial conditions. See S5 Text for details of model analysis.

Results

A Tipping Point between CRISPR and SM Strategies at High Viral Titres

We analyzed our model to find the long-term equilibrium outcome of competition between a laggy CRISPR-immune strain and a costly SM strain (S5 Text; $\kappa = 0.01$). Over a wide range of parameter values the model yielded a single CRISPR-immune equilibrium where the SM strain went extinct (figure 2). Only when there was extremely high flow of viruses into the system did we see an alternative outcome where the system settled in an SM-only state and the CRISPR immune strain went extinct. For short lag times ($\phi \geq 10^3$), the "tipping point" from an all-CRISPR to all-SM state occurred as the external viral pool (v_0) exceeded concentrations of 10^9 PFU/mL (figure 2). In no case did the two strains, CRISPR and SM, coexist stably over the parameter regimes considered. Thus our model predicts a sharp transition from a CRISPR strategy being favored to an SM strategy being favored at high viral titres. This is consistent with previous work on inducible immunity that saw a steep decrease in the relative fitness of a CRISPR-Cas immune strain when competed against an SM strain at very high viral titres [29].

Yet, previous experiments appear to disagree on the severity of the inducible cost of CRISPR-Cas immunity. In the original work on the topic, Westra et al. [29] observed a steep transition from high relative fitness (> 1) to a relative fitness of essentially zero for a CRISPR strategy competed

against an SM strategy in competition experiments with increasing MOI, consistent with our model's predictions (S5 Figure). More recently, Alseth et al. [30], did not observe this steep fitness decrease while performing nearly identical experiments. We suspected that these later experiments failed to capture the transition from high to low relative fitness seen in our model because they were not carried out to a sufficiently high viral titre. Therefore, we replicated the Alseth at al. [30] experiments with the same host-phage system, *Pseudomonas aeruginosa* UCBPP-PA14 and its lytic phage DSM3*vir,* out to a higher viral titre (10^{10} PFU/mL) and were able to capture the steep decrease in fitness of the CRISPR-immune strain at high MOI (figure 3), confirming our model predictions. We replicated these competition experiments *in silico* to more precisely illustrate this point (figure 3, red line). Our lag model captures the major shift from CRISPR-to-SM strategy that happens at high viral densities as seen by Westra et al. [29], consistent with the idea that immune lag causes the inducible cost of CRISPR-Cas immunity (figure 3, S5 Figure). Importantly, the original work by Westra et al. [29], showed that the inducible cost of CRISPR-Cas immunity was not due to virus-induced mortality, as even less-fit CRISPR-immune cells survived at high viral titres.

Finally, we note that while the qualitative results of these competition experiments are highly reproducible, with a steep decrease in the fitness of CRISPR-Cas immune strains occurring at high MOI, where exactly this transition occurs and the baseline relative fitness of the CRISPR-immune strain in the absence of virus appear to be quite variable between replicates and experiments (figure 3, S5 Figure). Viruses and host cells were quantified using serial dilutions, introducing the possibility of multiplicative errors and perhaps making cross-experiment variability less surprising. This cross-experiment variability prevented us from obtaining precise lag estimates (we estimate that $10^{-3} \leq \frac{1}{\phi} \leq 0.1$). Initial host density in particular can strongly affect model expectations (S6 Figure). For model results reported below we include an analysis of both short ($\phi = 10^3$) and long ($\phi = 10$) lag times to capture the full range of experimental variability.

Immune Lag Is Extremely Costly During an Outbreak of Novel Virus

We simulated an outbreak of "novel" virus to which preexisting CRISPR-Cas immunity did not exist in the population, or to which only a very small proportion of the population was already immunized. We found that during outbreaks of such "novel" viruses immune lag can be extremely costly, leading to selection for an SM defense strategy over a CRISPR strategy, even when the SM strategy comes with a growth cost. The cost of lag was only apparent when we examined the non-equilibrium dynamics of our model and is relevant to natural systems where outbreaks of novel or mutant viral strains may occur at moderate to high frequency. Unlike our results described above for systems at equilibrium, even a very low rate of immigration of novel viruses into the system can lead to a massive reduction in the fitness of a CRISPR-Cas relative to an SM strategy if most host are not already immunized.

We solved our lag system numerically starting from a dense resident susceptible population ($S = 10^8$), small populations of CRISPR-immune and SM host ($C = M = 100$) and a small environmental viral pool ($v_0 = 100$). During the resulting outbreak, as the viral population spikes early it suppresses the initial growth of CRISPR-immune host, leading the SM population to dominate (figure 4a-c). This initial dominance of SM even occurs when the cost of an SM strategy is very high (S7 Figure). The only way for CRISPR-immune host to dominate during an outbreak is for the duration of immune lag ($\frac{1}{\phi}$) to approach zero, but even short lags ($\phi = 10^3$) result in a substantial initial expansion of the SM population (figure 4b).

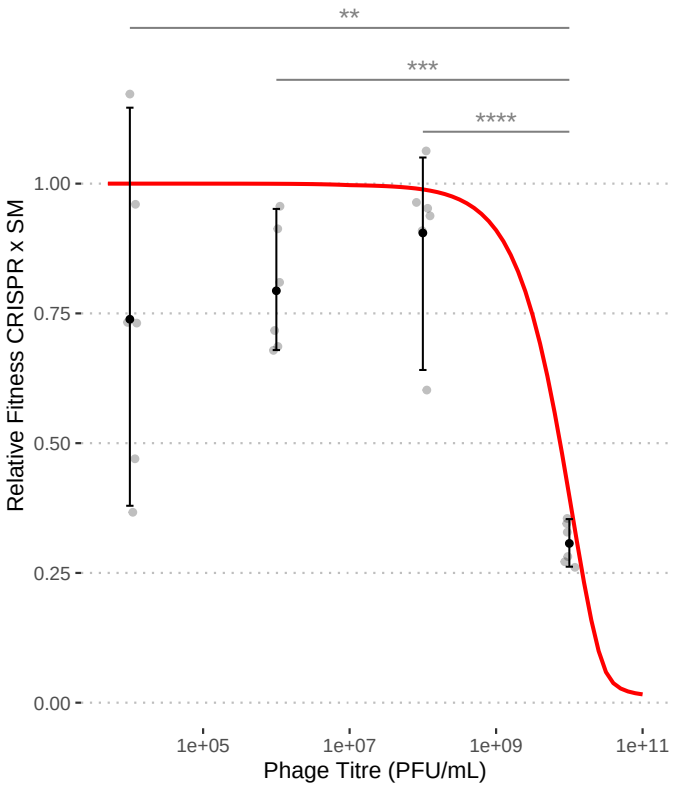

Figure 3: **A strongly inducible cost of CRISPR-Cas immunity at high viral titre is consistent with "laggy" CRISPR-Cas immunity.** (a) Competition experiments between BIM2 and SM strains in the presence of various viral titres. Fitness calculated based on densities one day post infection. Red line shows results from *in silico* competition experiments with no cost for SM ($\kappa = 0$) and a short lag time ($\phi = 10^3$) for comparison. Stars indicate significant differences between conditions (**, $p < 10^{-2}$; ***, $p < 10^{-3}$; ****, $p < 10^{-4}$; Tukey's Honest Significant Difference Test). These results qualitatively reflect the outcomes of similar previous experiments with the same strains (S5 Figure; [30, 29]), though that work suggests a much longer lag period ($\phi \approx 10$).

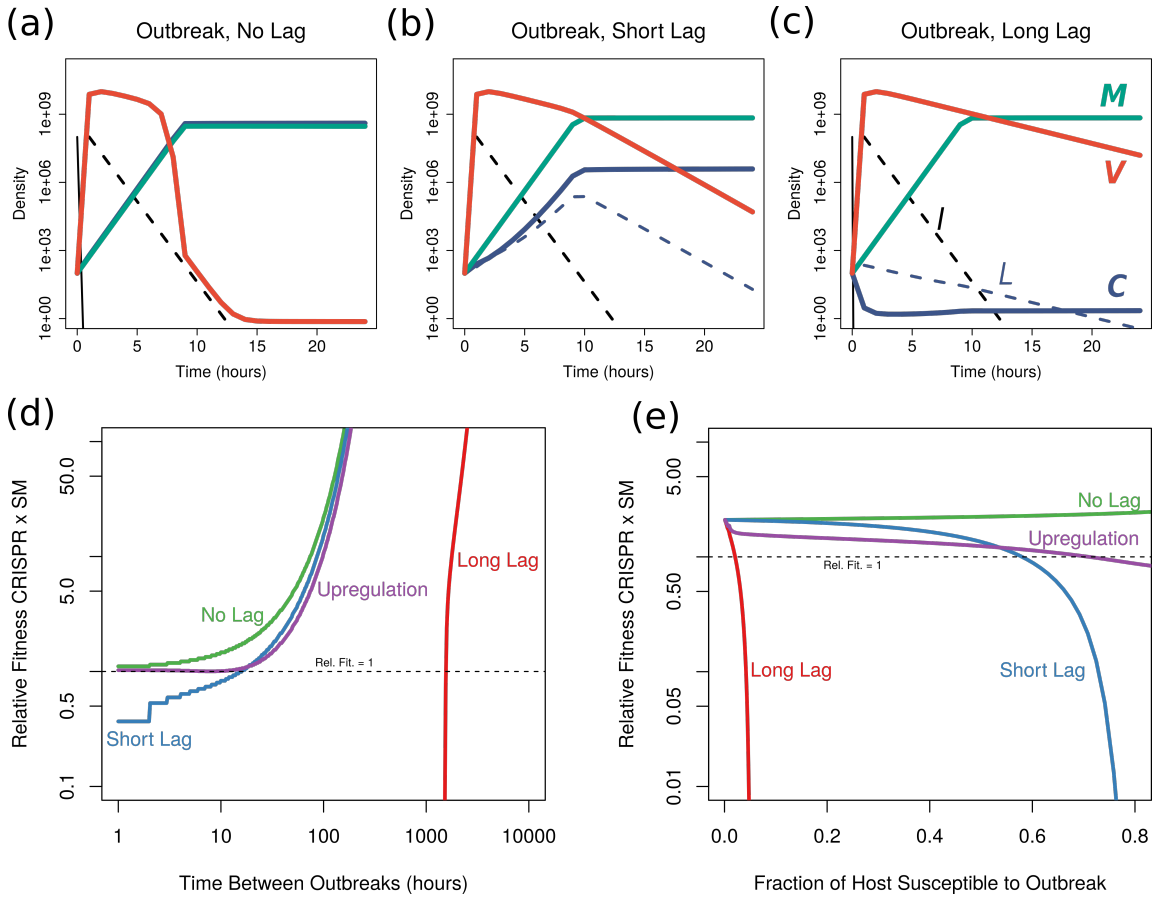

Figure 4: **Immune lag prevents the dominance of a CRISPR-immune (C) strategy over a costly SM strategy (M) during repeated outbreaks.** (a-c) Immune lag prevents CRISPR-Cas from out-competing a costly SM strategy ($\kappa = 0.01$) early on in an outbreak (starting from a dense susceptible population, $S = 10^8$). Numerical solutions for lag model with CRISPR-immune (purple), SM (green), viral (orange), and susceptible (black) populations shown. Note that as the system approaches equilibrium the CRISPR strain will eventually out-compete the SM population (though this may take a long time), regardless of lag. (d-e) Immune lag prevents the CRISPR strategy from out-competing an SM strategy when outbreaks are frequent. Results shown for iterated outbreak model with the size and frequency of outbreaks varied. Outbreaks of novel virus must be rare or affect a small fraction of the community for the CRISPR strategy to out-compete an SM strategy. "Short" and "long" lags correspond to the upper and lower bounds estimated for ϕ in figure 3 ($\phi = 10^3$ and $\phi = 10$, respectively). "Upregulation" refers to a system with a CRISPR-immune strain with a long lag ($\phi = 10$), but that can be upregulated to a "fast" immune state ($\zeta = 0.1$). In (e) outbreaks affect 50% of the host population (interval between outbreaks varied) and in (d) outbreaks occur every 24 hours (fraction of host population affected varied).

Even though an SM strategy will dominate immediately after an outbreak, if the SM strategy is sufficiently costly then the system will eventually return to a CRISPR-dominated equilibrium. How long will this return to CRISPR-dominance take, and what happens if the system is perturbed again before then? In natural communities, novel viral strains to which the host lacks preexisting immunity may emerge via mutation or immigrate into the system via dispersal. We found that even moderately frequent outbreaks can lead to selection against a CRISPR-based defense strategy.

We simulated our system's dynamics under repeated outbreaks at set intervals (see Methods), corresponding to either the emergence of an escape mutant in the viral population or the arrival of a novel viral species into the system against which the host lacks a preexisting spacer. We observe that for long lags ($\phi = 0.1$), if outbreaks occur even with moderate (monthly) frequency, immune lag will prevent a CRISPR strategy from rising to dominance (figure 4d). These results agree with empirical observations that the repeated addition of susceptible host into a host-phage system promotes the evolution of an SM strain over a CRISPR-immune strain [32]. Note that we assume that outbreaks affect both CRISPR and SM strains, so that novel virus can overcome both defense strategies, in order to compare strategies on an equal footing and calculate the precise cost of lag. In reality, the probability of a viral mutant escaping spacer targeting versus the probability of a viral mutant being able to target a new or modified host receptor are likely to be quite different, which would alter the frequency of outbreaks that host populations employing these two strategies would experience. In all likelihood, the respective rates of coevolutionary dynamics for hosts with CRISPR and SM strategies will vary a great deal across systems in complex ways that are difficult to capture with simple models.

CRISPR-Cas systems have one important advantage that our model neglects – different host may have different spacers, leading to a great deal of immune diversity in the population. This diversity is protective, as it makes it much more difficult for viral escape mutants to gain a foothold [61, 62, 63]. To account for host immune diversity, we varied the fraction of the host population susceptible to each novel viral outbreak (figure 4e). Even daily outbreaks affecting less than 10% of the host population will prevent the dominance of a CRISPR-Cas defense strategy when lags are long ($\phi = 10$). The spacer frequency distribution in host populations is often highly skewed, so that a few spacers are found among a large fraction of hosts [64, 65, 66]. Given that viral outbreaks are generally expected to affect the most abundant host sub-populations [67], in the presence of small, frequent outbreaks, such a skewed distribution of immune variants would make it very difficult for a CRISPR-immune host to outcompete an SM population.

Inducible Defenses Can Mitigate The Effects of Immune Lag

The *cas* operon, or a subset of *cas* genes, are often transcriptionally upregulated in response to infection or to conditions that indicate a high risk of infection [54]. We found that if strong upregulation occurs after infection, so that the resulting "fast immune" cells with an upregulated CRISPR locus do not experience lag, the overall effects of immune lag can largely be mitigated during an outbreak (figure 4d-e). This result is relatively robust to variations in the rate at which cells return to normal expression levels (ζ), though high rates of return will ameliorate lag less than lower rates (S9 Figure).

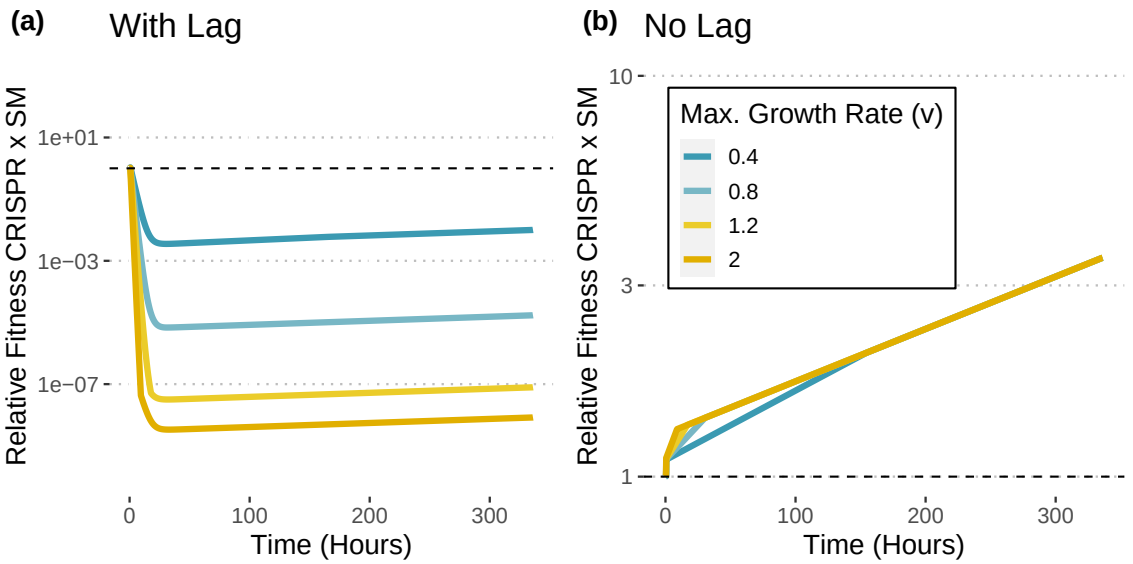

Figure 5: **Immune lag is less costly in systems with slow growth.** Change in relative fitness with respect to $t = 0$ during a viral outbreak in host populations with different maximal growth rates (v). (a) Relative fitness for the CRISPR-Cas immune strain competed against an SM strain drops off very steeply at the outset of an outbreak for faster growth rates (starting from a dense susceptible population, $S = 10^8$; long lag time, $\phi = 10$). The drop in relative fitness is less severe in slow growing host populations. (b) For a system without lag this pattern is flipped, with CRISPR-immune strains having higher relative fitness when growth rates are high.

Laggy Immunity is Less Costly in Slow Growing Hosts

It has been suggested that slow growing and less dense host populations will favor CRISPR-Cas immunity whereas fast growing, dense populations will favor alternative defense strategies (*e.g.*, SM; [27, 28]). Consistent with growth affecting the evolution of host defense strategy, Westra et al. [29] showed that high resource environments favored an SM strategy over a CRISPR strategy in direct competition experiments. We wondered if lag could partially explain this phenomenon. Using our lag model, we found that in host populations with a high maximal growth rate the cost of immune lag was much greater than in slower growing populations (figure 5). Thus, if CRISPR-Cas immunity is laggy, it is much more likely to be favored over an SM strategy if the host has a slow maximal growth rate. The opposite is true when CRISPR-Cas has no lag (figure 5). Intuitively, the temporary slow down in growth for host with a laggy immune systems is felt less strongly if growth is already slow, whereas the impact is much greater in a highly competitive system with fast growers.

Discussion

We built a biologically-motivated model of CRISPR-Cas immunity that links population-scale host-virus dynamics to molecular-scale changes within the cell. In doing so, we were able to demonstrate that immune lag can strongly impact the evolution of immune strategy in some prokaryotes [31, 29]. Immune lag's effect is felt most severely during an outbreak of novel virus. We showed that for even moderately frequent outbreaks of novel viruses that are unrecognized by the CRISPR-Cas immune system, immune lag will lead to selection against CRISPR-Cas in favor of other defense strategies (*e.g.,* surface modifications). Note that we are making an argument here about selective forces acting on immune strategy – the rate and mechanics of how CRISPR-Cas functionality might be lost in natural settings, as well as the implications of this loss, are outside the scope of this study and have been explored elsewhere [22, 39].

Even considering the beneficial effects of priming seen in many systems [10, 11], where partial spacer-protospacer matches stimulate rapid spacer acquisition thus allowing hosts to "update" their immune memory against viral escape mutants, it is not unreasonable to expect wholly novel outbreaks on a daily or weekly timescale for natural systems with high viral migration. That being said, primed adaptation can still help overcome short lags in the special case where outbreaks of novel virus affect the entire population of defended host (S3 Fig). Our results emphasize the benefits of having multiple redundant spacers towards the same target, as even temporary loss of immunity in a subset of the population can lead to strongly negative fitness effects for the entire immune host population due to lag induced by the resulting high-density viral bloom.

Immune lag has strong negative fitness effects even when CRISPR-immune strains are competed against very costly SM strains. In culture, SM strategies may occasionally be essentially cost-free, but in natural systems surface molecules that act as viral receptors often play an important role in host fitness, which can prevent the emergence of SM strains [30, 68]. Nevertheless, during an outbreak in a primarily susceptible population very costly SM strains still rise to dominance (S8 Figure). Thus, lag is likely to be relevant even in natural systems where surface modifications are very costly. Phage DMS3vir uses the host pilus as a receptor, meaning that SM mutants are defective in terms of motility, though we saw no great difference in fitness between SM and CRISPR strains in the absence of phage in our experiments (Fig 3; and see [29, 30, 31]).

Some immune host strains may be able to partially avoid the effects of lag. The impact of lag can be mitigated by transcriptional upregulation of the *cas* locus (figure 4). Thus lag may help explain why expression of the *cas* genes is tightly regulated in many systems [30, 31], in combination with other explanations such as avoidance of autoimmunity [69]. Additionally, lag seems to have less of an impact on slow growing host populations, perhaps explaining in part the suggested, though not yet systematically demonstrated, pattern in which CRISPR-Cas is more common among slow growing and low density taxa ([27, 28]; though of course many organisms capable of fast growth, including *Pseudomonas aeruginosa*, also have CRISPR-Cas). These variations may perhaps explain why the prevalence of CRISPR-Cas immunity varies so widely between different groups of organisms (*e.g.*, between anaerobes and aerobes [70], between bacteria and archaea [8, 71]).

We emphasize that expression of viral genes and/or changes in host expression are not the only phenomena that could lead to a slowdown in the growth of immune host upon infection. For example, membrane depolarization due to viral injection could lead to a transient growth slowdown. Our model is agnostic to the mechanism causing lag, and only requires that two criteria be satisfied: (i) the virus-induced fitness reduction is due to growth inhibition rather than increased mortality (based on Westra et al. [29]), (ii) the virus-induced fitness reduction is felt only by cells that allow

for viral entry (*i.e.,* not SM cells). That being said, in the experimental system we consider, Meaden et al. [31] provide strong evidence that the transient expression of viral genes leads to a reduction in the fitness of immune host.

Finally, it is clear that the severity of immune lag is a shared trait determined by both host and virus. Different viruses reprogram the cell in different ways and to different degrees (*e.g.,* [35]). Similarly, hosts will likely vary in their susceptibility to reprogramming. Our overall conclusions are relatively insensitive to this variability, as even short lags can have a severe impact on host fitness during an outbreak (S7 Figure). Nevertheless, this variability makes it difficult to put realistic bounds on our lag parameters, and as we show in figure 4, upregulation of the CRISPR locus may mitigate immune lag for the host [54]. For other types of intracellularly-acting defense, such as abortive infection systems in which infected host cells do not recover, lag may be irrelevant. In any case, we highlight an important parameter of virus-host dynamics, the recovery rate of defended cells to viral infection (ϕ), that is not typically measured or considered in theoretical treatments of host-phage systems. This parameter is likely universal to all intracellularly-acting DNA- or RNA-degrading defense systems, including restriction-modification systems, which are nearly ubiquitous [72]. Immune lag is quite possibly a widespread phenomenon common to many classes of defense systems acting within the cell (*e.g.,* [73, 74, 75]), and deserves consideration in any population-level study of prokaryotic antiviral defenses.

Methods

All code and raw data necessary to run models and generate figures available at `https://github.com/jlw-ecoevo/immunelag`. See S5 Text for model analysis.

Competition Experiments

For the competition experiment shown in figure 3, a *Pseudomonas aeruginosa* UCBPP-PA14 bacteriophage-insensitive mutant with two CRISPR spacers in a type I-F system (referred to as BIM2), a surface mutant (loss of the Type IV pilus) derived from the PA14 *csy::LacZ* strain, and the *P. aeruginosa* specific lytic phage DMS3*vir* were used. Prior to experiment start, bacteria were grown for 24 h in glass microcosms containing 6 ml LB medium, being incubated at 37°C while shaking at 180 r.p.m. The two PA14 phenotypes were competed against each other for 24 hours in the presence of phage DMS3*vir* at either 10^4, 10^6, 10^8, 10^{10} PFU/ml ($n = 6$ per treatment) while incubated under the same conditions as described above. Bacteria were sampled at timepoint 0 and 24 h post phage infection, before being diluted in M9 salts and plated out on LB agar. The agar was supplemented with ca. 50 µg mL^{-1} X-gal to differentiate between the BIM2 and surface mutant. Bacterial colonies were counted, after which relative fitness was calculated as previously described [29].

Simulating Competition Experiments

We modify our model to take place in serial transfer, where the change resource density is described by a constant decay over time until the next transfer

$$\dot{R} = -\frac{evR}{z+R}(S+C+M) \tag{7}$$

and the washout terms are omitted from equation (1). Based on an equilibrium host density in LB of $\sim 10^9$ CFU/mL, we estimate an initial resource density at each transfer of $R(0) \approx 500$ μg/mL. We initialize the experiment with with a density of 10^7 CFU/mL of CRISPR-immune and and SM host (no susceptible) and solve our system numerically at 24 hours. Viruses are either added at varying initial densities up to 10^{11} PFU/mL. System was solved numerically using "lsoda" solver in the deSolve R package [76].

Simulating Intermittent Outbreaks

To model the effects of intermittent outbreaks we solved our ode system with immune lag in a chemostat/virostat ($v_0 = 100$). The outbreak interval was set at some length τ, and the system was solved numerically to this point (using the "lsoda" solver in the deSolve R package [76]). The solver was then paused, and the identity of the viral pathogen flowing into the system was changed to a "novel" strain able to infect some fraction f of previously immune or resistant hosts (implemented by letting $S = f \times (C+L+M)+S$, $C = (1-f) \times C$, $L = (1-f) \times L$, and $M = (1-f) \times M$). This was repeated for 10 iterations of length τ and relative fitness was calculated by comparing initial to final densities of CRISPR-immune and SM populations. Importantly, we let both CRISPR-immune and SM populations experience the outbreak in order to directly compare the ability of these strategies to deal with repeated infection. This setup is similar to the experiments performed by Chabas et al. [32] who found similar results in an empirical system. We also neglect initial immunization dynamics by starting the population in an immune state ($C(0) = M(0) = 5 \times 10^7$) at the beginning of our simulations. For figure 4d we let $f = 0.5$ and for figure 4e we varied f.

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Data Accessibility

All code and raw data necessary to run models and generate figures available at `https://github.com/jlw-ecoevo/immunelag`.

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