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Viral Population Dynamics and Virulence Thresholds

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Abstract

Viral factors and host barriers influence virally induced disease, and asymptomatic versus symptomatic infection is governed by a "virulence threshold". Understanding modulation of virulence thresholds could lend insight into disease outcome and aid in rational therapeutic and vaccine design. RNA viruses are an excellent system to study virulence thresholds in the context of quasispecies population dynamics. RNA viruses have high error frequencies and our understanding of viral population dynamics has been shaped by quasispecies evolutionary theory. In turn, research using RNA viruses as replicons with short generation times and high mutation rates has been an invaluable tool to test models of quasispecies theory. The challenge and new frontier of RNA virus population dynamics research is to combine multiple theoretical models and experimental data to describe viral population behavior as it changes, moving within and between hosts, to predict disease and pathogen emergence. Several excellent studies have begun to undertake this challenge using novel approaches.

Virulence thresholds in mixed viral populations

Recent work with poliovirus demonstrated that the presence of a pathogenic virus within a non-pathogenic population is not necessarily enough to establish a virulent phenotype. In fact, the attenuated virus protected against disease, demonstrating the presence of a virulence threshold [1]. For mixed viral populations, viral and host barriers control the threshold and determine symptomatic versus asymptomatic disease. For poliovirus, even a virulent virus with enhanced fitness and replicative capacity was unable to overtake the mixed population and cause disease in the presence of an intact immune system. This review will discuss viral population dynamics in the context of virulence thresholds highlighting new and previously identified viral and host barriers and how they impact viral population dynamics and pathogenesis.

Quasispecies models and principles governing viral population dynamics

Quasispecies theory was developed as a mathematical model to describe features of replicative systems within evolutionary biology [2]. The term quasispecies has since been adopted as a framework to model viral population dynamics, specifically for those viruses whose generation is characterized by high viral yields and replication via an error prone

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polymerase. Examination of quasispecies theory through viral dynamics research has yielded a lucrative interaction between evolutionary theory and experimental evaluation of the resultant models through viral systems [3,4].

We can now use quasispecies theory and modeling of viral dynamics to attempt to predict disease in the context of virulence thresholds. For viruses the term quasispecies refers to a mutant swarm of related but genetically distinct population members. This population is in dynamic flux, continually subjected to competition, host pressure, selection, and genetic variation [5]. Artificial quasispecies can be generated to test modulation of virulence thresholds in the presence of these viral and host pressures [1,6,7]. Experimental evidence has uncovered a few unifying principles of viral dynamics as predicted by population modeling and quasispecies theory. First, it can be argued that RNA viruses have evolved the perfect error rate, where too many mutations result in error catastrophe and collapse of the parental genome sequence and viral extinction, and too few mutations result in a viral population unable to cope with selective pressure [8,9]. Experimentally this has been demonstrated using mutagens where high mutation rates lead to accumulation of detrimental mutations and population extinction but high fidelity escape variants yield a population with decreased diversity. Both “over-mutagenesis” and “under-mutagenesis” can attenuate viruses *in vivo* and *in vitro* [8-11]. Therefore, error rate contributes to the virulence threshold and alteration of error rate can be used to attenuate the population and limit disease [10-12].

Second, the importance of genetic diversity in the viral population has been demonstrated in multiple systems including plant viruses, phage, mammalian viruses, aquatic viruses and arboviruses. Bottleneck effects are observed when the population undergoes host imposed pressure or restriction due to viral population interactions [13-15]. For example, a viral population can enter a host as a diverse quasispecies and upon encountering a host barrier or selective pressure, only a few variants may have a genotype that allows escape. Once the variants evade the pressure, they may then replicate to high yields. The resultant population has decreased diversity until expansion and mutation accumulation restores diversity.

A third principle influencing the virulence threshold is fitness. The term fitness refers to replicative capacity in a given environment and can be used to describe an individual pool member, phenotype of a subgroup of the population, or the population as a whole. The effects of fitness have been explored experimentally through competition between variants and these experiments have generated multiple sub-principles that describe fitness effects in different viral systems and different environments. A diverse population can demonstrate increased fitness, since variants can be present within the population that are able to replicate in restrictive environments [11]. Conversely, adaptation to a single host has been proposed to increase fitness. This idea, the “trade off hypothesis”, suggests that adaptation in one host creates a fitness cost in another host. Depending on the system there are conflicting reports for these effects [16-20]. Arboviruses show decreased mutation accumulation rates compared to RNA viruses replicating in one host and it has been argued that this is due to continual host switching. However, often experiments have failed to validate this theory. For West Nile virus it has been shown that the tradeoff hypothesis is supported for adaptation in the bird host, but not in the mosquito [16]. Conversely, for dengue virus, it was demonstrated that release from host alternation facilitates adaptation but does not necessarily result in a fitness tradeoff in the bypassed host [21]. The argument could be made that both generalists (capable of infecting a variety of host species) and specialists (infecting one or few host species) have an advantage depending on the viral system tested, the environment, and the evolutionary history of the founding population [18,20,22]. In each unique instance there is a virulence threshold for disease and any environmental fluctuation or alteration of

the virus genome or population can shift the threshold causing more or less symptomatic disease.

The dynamic nature of highly evolvable viral populations renders some counterintuitive models of evolutionary theory plausible. Viruses have been described as moving through varying fitness landscapes where peaks represent high-fitness genotypes and valleys represent low-fitness genotypes [7,18,23]. “Survival of the flattest” is a fitness landscape model proposing that under high mutation rates robust genomes of sub-maximal fitness are favored over fast homogeneous replicators with the highest fitness [24-26]. Robustness is resistance to phenotypic change due to mutation, relative to parental sequence. Mutation selection balance via increased robustness has been suggested, but experimental evidence has generated conflicting reports for different viral systems. Using bacteriophage ϕ6, genetic robustness was shown to increase evolvability of thermotolerance [27]. For vesicular stomatitis virus it was demonstrated that high-fitness variants evolved under bottlenecked conditions showed lower robustness [17]. However, experiments with LCMV failed to find mutants with decreased sensitivity to a mutagen, and the authors suggest that modulation of fecundity requires time to acquire multiple mutations to change robustness phenotypes.

One explanation for varying results is that incongruent fitness landscapes in different environments impact RNA virus population dynamics more than tradeoffs [28,29]. Using VSV it was demonstrated that most viral populations had increased fitness in all environments tested in the absence of a fitness decrease in bypassed environments [18]. This study further demonstrated that differences in fitness could be due to slow increases in fitness in a new environment rather than fitness reductions. Taken together these experiments demonstrate progress made using evolutionary biology to define, elucidate, and validate basic principles of viral population dynamics and virulence thresholds. The challenge now is to apply these principles to understand how the viral population changes as it moves within and between hosts to develop complete pictures of spatio-temporal regulations and fluctuating host environments.

Viral dynamics within and between hosts

Several groups have begun to apply unifying rules to predict outcomes of therapeutics, vaccines, and pathogen emergence taking into consideration virulence thresholds and factors that influence how the viral population changes as it moves through sequence space, time, and new environments. Three themes have emerged as important in determining viral dynamics: population size, the effects of variable multiplicity of infection (MOI), and differential replication modes. Not surprisingly, it has been demonstrated that population size or viral load predicts disease [30]. However, how population size changes and influences disease as viruses move within a host and between various tissues needs further elucidation. Host barriers limit pathogenesis and restrict viral load differentially in various tissues [30,31]. For poliovirus it has been demonstrated that the type I interferon response plays a role restricting viral replication in peripheral tissues, but not necessarily in the central nervous system where inefficient retrograde axonal transport may be the primary factor limiting transport and replication [6,32]. Similar trends exist for multiple viral systems where host restriction of a viral population changes during dissemination and transmission [13,31,33,34]. A recent report proposes treating viruses as multiple subpopulations that colonize and replicate in different niches rather than homogenous populations [35]. The authors struggled to find genotypes responsible for virulence phenotypes and suggest hidden virulence determinants exist due to population dynamics. This finding was further supported by a HIV-1 fitness recovery study, where quasispecies heterogeneity was responsible for increased replicative capacity in the absence of modifications to the consensus sequence [36].

In order for the viral population to exert effects that cannot be accounted for by the genome sequence there is a dependence on density and differential MOI to drive competition and selection [37,38]. Host barriers restrict viral populations and the effect of these barriers is impacted dramatically by MOI [39]. It has been demonstrated that MOI influences evolution and competition but this varies as viral populations disseminate within a host [28]. The initial cycle of replication is arguably the most influential during a viral infection. This first replication cycle frequently occurs at a low MOI where the fitness of individual variants will determine subsequent dissemination. A recent paper using *Cauliflower mosaic virus* (CaMV) to analyze the dynamics of MOI during dissemination found that during the initial infection MOI was low, but MOI subsequently increased after viral replication and dissemination. However, infection returned to low MOI conditions during later stages [28]. The return to a low MOI is intriguing because low MOI conditions can favor development of a more diverse and fit population to aid transmission to a new host. When MOI is high and fixed, virus yield can rapidly decline [40,41]. Perhaps this mechanism has evolved for the viral population to modulate virulence to balance the threshold for disease and optimal transmission. A theory for the evolution of segmented genomes has been proposed based on the ability of defective interfering (DI) particles to complement one another. It was shown experimentally that packaging of shorter genomes increased particle stability suggesting a critical MOI range for the evolution of segmented genomes [42]. Perhaps the intrahost fluctuations in MOI drive viral evolution to balance particle stability, fast replication, diversity, and increased fitness to ultimately aid transmission.

A long-standing mystery is why most virions in a population are non-infectious, thus generating high particle to PFU ratios. DI particles are thought to contribute to this effect [40,41,43-45]. DI particles have an effect on pathogenesis because recombination, competition, and complementation, which drive viral diversity and evolution, can occur among viral genomes and DI particles during high MOI conditions [42,46]. Complementation influences error threshold [45], and can facilitate compensatory evolution for gene deletion to operate beyond is its functional network [47]. The effect of deleted genes within individual viral genomes can be rescued by trans interactions with gene products from co-infecting particles. These complex intracellular interactions may explain why population phenotype cannot always be explained by viral genotype [48].

A final consideration when generating models of colonization dynamics is how different replication modes across spatial constraints influence infection outcome [25,49]. A model accounting for differential modes of viral replication was recently developed and considers spatial constraints to dissemination [49]. Although complex, the authors were able to apply their findings to different viral systems. For example, they demonstrated a particular mode of replication and spatial constraints yielded increased robustness associated with superinfection limitation consistent with what has been observed for plant viruses [49]. These new complex models account for population density, variable MOI, differential replication modes, and spatial constraints to understand dynamic viral systems. Once modulation of virulence thresholds as the viral population moves throughout and between hosts is understood, it may be possible to predict pathogen emergence and rationally design therapeutics.

Virulence thresholds, weighted modeling, and population movement

During natural infection susceptible hosts and cells are stochastically encountered; therefore, an organizational framework to understand stochastic events impacting viral infection would be invaluable. One approach already being taken to develop, expand and validate complex models of viral population dynamics is to weight probable outcomes based on experimental observations and principles already vetted. For example, initial infection in plant viruses

may be more restricted than animal viruses, yet transmission may be easier. This phenomenon is now possible to investigate using a new assay for vector transmission of viruses using live plant cells and an aphid membrane-feeding assay [50,51]. Similarly, evolution could be low for arboviruses under low MOI conditions but high during infections with an elevated MOI environment. The dynamics of MOI fluctuation can be elucidated using ultra-deep sequencing approaches to uncover the composition of sub-populations during viral infection [52-54]. Additionally, it has been shown that arboviruses require diversity and sequential adaptive mutation for fitness and host switching which could inform predictions of novel pathogen emergence or re-emergence [9,55,56]. It has been demonstrated that mutational robustness of coxsackievirus B3 is less than poliovirus, enhancing sensitivity to lethal mutagenesis, thus influencing therapeutic efficacy [26]. Foot-and-mouth disease virus requires multiple steps to adapt to mutagens but ultimately can escape extinction [57]. These concepts could inform rational therapeutic design for different viruses.

Using the principles discussed above, factors that influence the population at various stages of infection and transmission can be assimilated and weighted differentially to gain a complete picture of virulence thresholds during infection and transmission (Figure 1). Spatiotemporal effects and population dynamics in various ecological compartments must be taken into consideration. Counterintuitive effects should also be accounted for. A tradeoff exists as described by Bull and colleagues [58,59], where the interaction between virus and host is driven towards an equilibrium between virulence and transmission. As parasites evolve with a host, reproductive capacity is increased, but extreme virulence is rare since transmission costs prevent the fixation of virulent phenotypes. In other words, if a virus is “too virulent”, debilitating the host, transmission is impeded and the virus will be eliminated through natural selection. DI particles have been shown to contribute to this phenomenon [1,37,60-63]. Therefore, as the viral population moves through the host, consideration should be made for how different host and viral factors modulate the virulence threshold within individual tissues to influence fitness and pathogenesis.

Incorporating virulence thresholds into predictions of viral population behavior can provide an additional dimension to quasispecies dynamics in that virulent viruses must be present above a certain proportion in order to cause disease, and this threshold is modulated by viral and host factors. Understanding modulation of virulence thresholds could lend insight into disease outcome in the presence of different viral populations in different hosts and aid in rational therapeutic and vaccine design.

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Highlights

- RNA viruses exist as dynamic populations of related genomes
- Viral population dynamics and host barriers influence virulence thresholds
- Understanding virulence thresholds may aid disease prediction

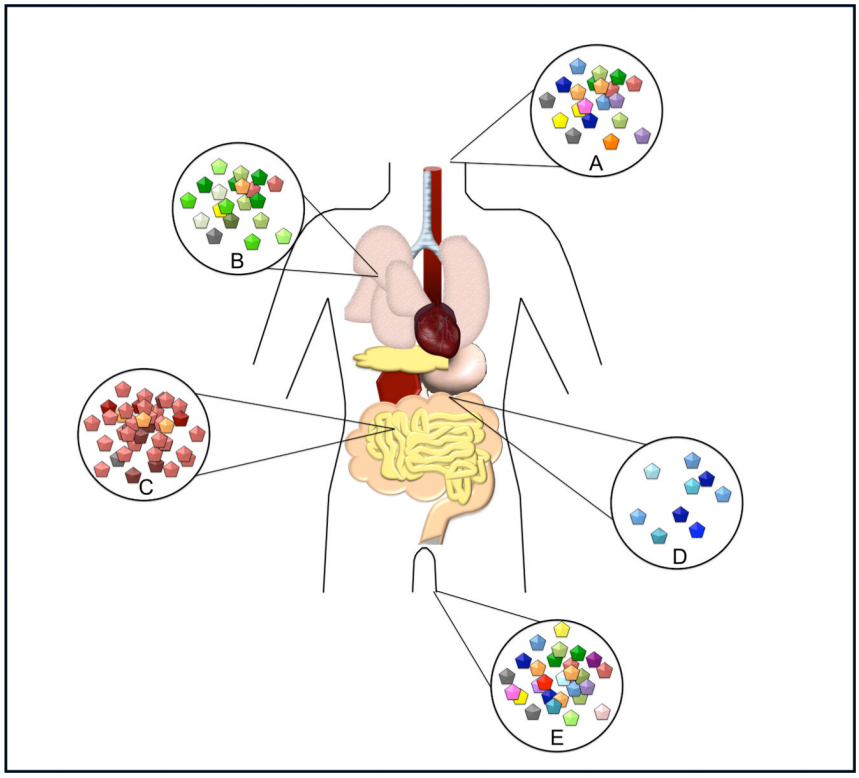

Figure 1. Model of viral population dynamics during intra-host and inter-host spread

A. In this case, the viral population enters the host as a diverse quasispecies with sufficient titer to establish infection. B. After entry the population may encounter a host barrier that limits diversity. Pool members with the ability to overcome this barrier may then replicate and restore diversity. The subsequent viral population in this tissue may have high diversity, but differ in overall consensus sequence from the initial infecting population. C. Certain tissues may be highly permissive for viral infection with limited host or viral pressures. The viral population in these tissues may become dominated by more fit variants with enhanced replicative capacity, resulting in high titer and low diversity. D. Alternatively, the viral population may enter a highly restrictive environment where host and viral factors limit productive replication. The resultant population may have decreased diversity and titer or be eliminated from this tissue entirely. E. A productive infection in a host should allow efficient replication in one or more tissues facilitating transmission of a viral population at high titer and high diversity to optimize transmission.