Effect of resource supply rate on host-pathogen dynamics

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ABSTRACT

The dynamics of model host cell (*E. coli*) and model pathogen (bacteriophage) populations were studied in chemostats with different resource supply rates. Resource supply rate was manipulated by altering the concentration of the limiting resource (glucose) in the incoming media. Population responses to increased resource supply rate were influenced strongly by the vulnerability of the host cells to infection. When the host cell population consisted entirely of cells equally vulnerable to infection, both pathogen and host cells responded to increased resource supply rate with an increase in their average densities. In contrast, when the host cell contained some cells that were less vulnerable to infection (i.e., partially phage-resistant *E. coli*), only the pathogen population responded to increased supply rate with a significant increase in average density. Furthermore, when the host cell population contained some cells completely invulnerable to infection (i.e., phage-resistant *E. coli*) only the host cell population responded to increased supply rate with an increase in average density. These responses were in general agreement with the predictions of mechanistic models of resource-consumer interactions.

Introduction

The development of disease is essentially an ecological phenomenon, a phenomenon that involves the ecological processes of invasion, competition, and consumption. The application of ecological theory to disease therefore has the potential to greatly increase our understanding. Although ecological models have long been used to describe the interactions between hosts and pathogens on the level of populations [17], it has only been recently that ecological theory has been applied to within-host interactions [15]. By thinking of the host as an ecological system, important insights emerge.

The role host nutrition may play in the development of disease is of increasing interest [7] and it is an area of research in which ecological theory may prove very useful [21]. Host nutrition can affect disease development through a number of potential mechanisms. Host nutrition can influence general mechanisms involved in the host's ability to respond to challenges by pathogens, for example, by maintaining immune system competence. Host nutrition can also affect disease dynamics through specific mechanisms such as within-host resource competition. For example, V. H. Smith and colleagues used resource-ratio theory to predict the dynamics of cells and pathogens *in vivo* [19-21]. They demonstrated that when cells and pathogens share nutrient requirements, the effect of host nutrition on the outcome of infection is consistent with the predictions of resource-ratio theory.

Theoretically, host nutrition can influence pathogen dynamics even when pathogens and cells do not share nutrient requirements. By affecting the growth of host cells, host nutrition could indirectly influence the dynamics of pathogens. For example, if host cells

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are limited by host nutrients, increasing host nutrients could increase the growth rate of cells, which could influence the size of a pathogen population feeding on these cells. Such indirect interactions have long been described in other ecological systems [8, 16] but have not previously been described in cell-pathogen systems.

Such indirect interactions can, theoretically, be influenced by a number of factors. For example, heterogeneity in cell vulnerability to infection can influence the response to increased nutrients. The indirect effect of host nutrients on pathogen dynamics is predicted to be very different when the cell population is completely vulnerable to infection versus when the cell population contains some individuals that are less vulnerable or invulnerable to infection. When cells are completely vulnerable, ecological theory would predict that an increase in the resources available to cells would result in an increase in the average size of a population of pathogens feeding on these cells. In contrast, the cell population is not predicted to increase in average size; it will turn over faster in response to increased nutrients but this extra production will be consumed by the pathogen population [3]. However, if some cells are less vulnerable to infection both pathogen and cell populations are predicted to increase as cell resources are increased [2]. The less vulnerable cells will increase at the expense of the more vulnerable cells, resulting in a decrease in the ratio of vulnerable to less vulnerable cells. Furthermore, if some of the cells are completely invulnerable to infection, the cell population is predicted to increase in average abundance while the pathogen population is predicted to remain unchanged in average abundance [4]. This increase in the size of the cell population is predicted to occur only through an increase in the number of invulnerable cells; the vulnerable cells are predicted to remain unchanged in average abundance. Cell vulnerability is thus predicted to have a significant effect on the response of cells and pathogens to host resources.

In this report, I review recent research by Richard Lenski and myself [2-4] in which we tested these models using laboratory populations of bacteria and bacteriophage. Cell resources and cell vulnerability were simultaneously manipulated in these experiments, and the observed dynamics were compared with the predictions of the ecological models. These studies demonstrate that the response of cell and pathogen populations to increased resources is significantly altered by heterogeneity in cell vulnerability.

Methods

Bacteria and phage are excellent model organisms with which to study cell and pathogen dynamics. More is known of the biology of phage-bacteria interactions than is known about most other cell-pathogen interactions; in addition, bacteria and phage are easy to cultivate and manipulate in the laboratory, and they share many of the basic attributes of other cell and pathogen systems. Our experimental system consisted of *E. coli* B strain REL607 [14], *E. coli* B strain REL6584 [4], and either the virulent bacteriophage T4 or T2 in glucose-limited chemostats. REL6584 is identical to REL607 with the exceptions that it is completely invulnerable to infection by bacteriophage T4, it is less vulnerable to infection by bacteriophage T2 and it cannot utilize the sugar arabinose. The ability to utilize arabinose has been previously shown to confer neither a competitive advantage nor disadvantage in a glucose-limited environment [12, 14]; we confirmed the neutrality of this marker in our experimental system [3]. We used this trait as a neutral marker to distinguish the two *E. coli* strains.

Decreased vulnerability to phage T4 and/or T2 has been shown to result in a competitive disadvantage relative to wild-type in a glucose-limited environment [10, 11].

We measured this disadvantage by co-inoculating REL6584 and REL607 into phage-free, glucose-limited chemostats and tracking their respective population densities. We calculated the competitive disadvantage as described by Lenski and Levin [13]. The disadvantage was approximately 35% for REL6584.

Using these strains, we assembled food chains of resource (i.e., glucose), host cells (i.e., bacteria) and pathogens (i.e., phage) that varied in the vulnerability of the cell population. Food chain A contained a cell population that was completely vulnerable to the pathogen (*E. coli* strain REL607 and phage T4). Food chain B contained a cell population that had both vulnerable individuals and less vulnerable individuals (REL607, REL6584 and phage T2). Food chain C contained a cell population that had both vulnerable and completely invulnerable individuals (REL607, REL6584 and phage T4). These food chains were maintained in chemostats for over 200 hours. We manipulated resource supply rate by using replicate chemostats into which was flowing media that contained different concentrations of glucose. The media consisted of Davis minimal broth [6] supplemented with 2×10^{-3} µg thiamine hydrochloride per ml and either 0.1 or 0.5 µg per ml glucose. At least three replicate chemostats at each glucose concentration were maintained simultaneously. Control chemostats without pathogens were established at each glucose concentration and maintained simultaneously with the treatment chemostats.

The population densities of the cells and pathogens were estimated twice daily by dilution and plating. REL607 cells were plated on Davis minimal agar supplemented with 2×10^{-3} µg thiamine hydrochloride per ml and 4×10^{3} µg per ml arabinose (this media allows growth of REL607 but not REL6584, since REL6584 cannot utilize arabinose). Heat-killed REL607 cells were mixed with each sample to inactivate free phage prior to plating, as described by Carlson and Miller [5]. Bacteriophage T4 and T2 were each plated on a lawn of REL607 using Davis minimal agar and the plate count technique described by Carlson and Miller [5]. REL6584 cells were plated on Davis minimal agar supplemented with 2×10^{-3} µg thiamine hydrochloride per ml and 4×10^{3} µg per ml glucose. A concentrated phage T4 lysate was mixed with each sample to kill REL607 cells prior to plating.

To estimate the average population densities of pathogens and cells, we treated each chemostat as a single observational unit. We first calculated the mean of the pathogen and cell population densities over time for each chemostat. We then estimated the average density of each population as the grand arithmetic mean of population density across replicate chemostats.

We compared average population density between the resource treatments with t-tests. One-tailed comparisons were performed because mathematical models of the food chains made directional predictions [2-4]. Prior to comparison we tested for homogeneity of variances. The data were log-transformed prior to comparison whenever the variances were found to be significantly different.

In past laboratory studies, the vulnerability of *E. coli* to infection by T2 and T4 has been observed to change due to evolution [10, 13]. Therefore, in each chemostat we tracked the evolution of mutants invulnerable to infection by T2 and T4. We estimated the total population density of mutants invulnerable to either T2 or T4 in each chemostat by mixing concentrated lysate of the appropriate phage with an aliquot of each chemostat sample and plating on minimal glucose media. We excluded from our analyses of population densities all time points that occurred after the detection of invulnerable mutants in the chemostats.

Results

Cell vulnerability had a significant effect on the response of host cells and pathogens to increased resources (Figure 1). The average density of pathogens increased strongly and significantly in response to increased resources when cells were homogeneously vulnerable (t = 18.225, df = 4, one-tailed $P < 0.0001$) and less strongly but nonetheless significantly when the cell population contained some less vulnerable cells (t = 14.836, df = 6, one-tailed $P < 0.0001$; Figure 1A). However, the pathogen population *did not* increase significantly when the cell population contained some invulnerable individuals (t = 1.2421, df = 4, one-tailed P = 0.1410; Figure 1A). The host cell population increased in response to increased resources weakly but significantly when cells were homogeneously vulnerable (t = 2.4699, df = 4, one-tailed $P = 0.0345$; Figure 1B). The cell population did not increase significantly when the cell population contained some less vulnerable cells (t = 0.2262, df = 6, one-tailed $P = 0.4143$; Figure 1B); however, the ratio of vulnerable to less vulnerable cells did decrease significantly (t = 2.1620, df = 6, one-tailed $P = 0.0369$). The cell population increased strongly and significantly when the cell population contained some invulnerable individuals (t = 15.238, df = 4, one-tailed P < 0.0001; Figure 1B). This increase consisted of an increase in the invulnerable component of the population only. All populations persisted in all chemostats with the exception of the high nutrient treatment of food chain B (the chain with some less vulnerable cells). In these chemostats, the more vulnerable cells declined over time.

Discussion

Ecological theory predicts that cell vulnerability will strongly influence the response of host cell and pathogen populations to increased cell resources. Our experimental results confirm these predictions. We found that laboratory populations of cells and pathogens responded differently to increased cell resources depending on whether the cell population was homogeneously vulnerable, had some less vulnerable members, or had some individuals that were invulnerable. In general, our observations of laboratory populations were consistent with theoretical predictions. However, there were two notable exceptions. First, we observed a slight increase in the cell population in response to increased resources when the population was homogeneously vulnerable; ecological theory predicted that the population would remain unchanged. The most likely explanation is that this difference is due to the spatial heterogeneity present in our system [3]. Recent research [18] suggests that even in well-mixed chemostats, growth of *E. coli* on the vessel wall can shelter *E. coli* cells from infection by bacteriophage. The presence of such a physical refuge from infection could explain the slight increase in the average density of the cell population at higher resource inputs. Second, we observed that the cell population did not significantly increase in response to increased resources when the cell population contained some less vulnerable individuals, contrary to the theoretical predictions. This may be due to the fact that the populations in this experiment were not at equilibrium (i.e., the more vulnerable cells were declining in density during the experiment). However, we did see a significant decrease in the ratio of vulnerable to less vulnerable cells in this experiment, as predicted by theory.

Cells and pathogens *in vivo* share many of the basic attributes of our model system and thus the potential exists *in vivo* for the type of interplay between host resources and cell vulnerability that our models predict. Whether this interplay actually occurs in a

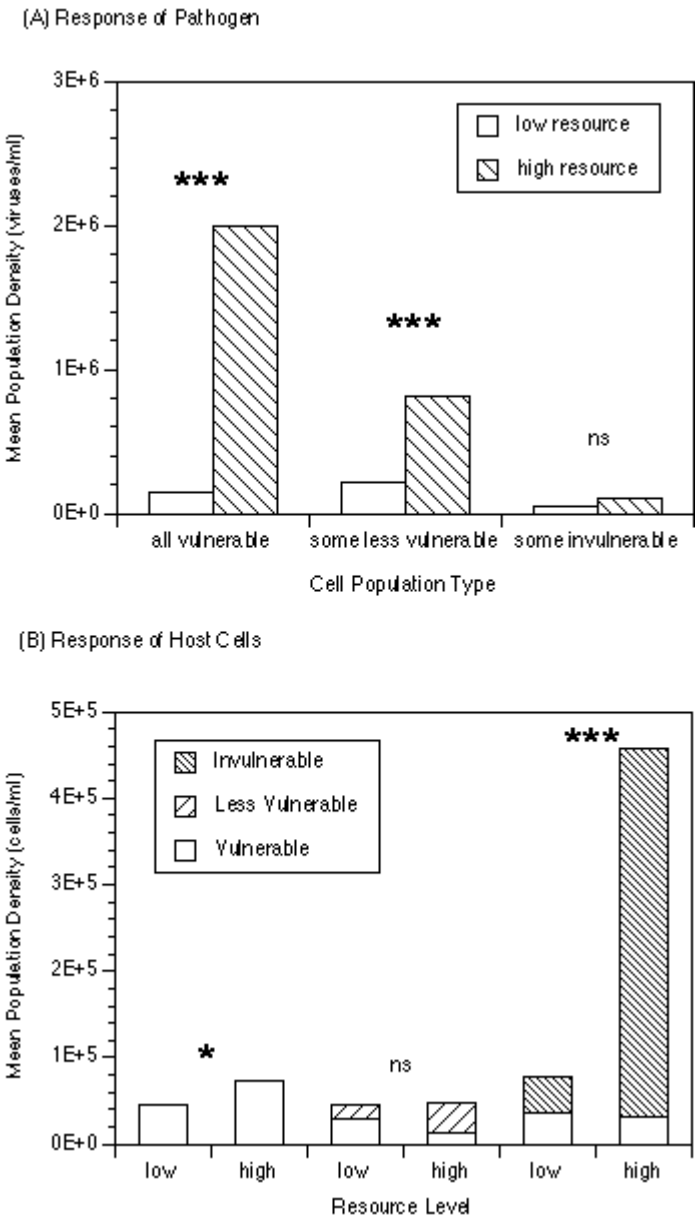

Fig. 1. Effect of increased host resources on the average density of pathogens and host cells. (A) average density of pathogens, (B) average density of host cells. Low resources = 0.1 µg glucose per ml, high resources = 0.5 µg glucose per ml.

particular cell-pathogen system depends on how well the particular system meets the assumptions of our models. For example, our models assume that host cells are self-reproducing and that cell reproduction is limited by nutrients. It is unclear how well this assumption is met *in vivo*. It is known that some mammalian cells are not self-reproducing, that some reproduce rarely and that others reproduce continuously. The rate of cell reproduction *in vivo* may be controlled by a number of factors, including host nutrition [1]. If cells are not self-reproducing and if cell reproduction is not at least partly limited by nutrients, then the type of interplay between host nutrition and cell vulnerability predicted by our models may not occur. Another important assumption of our models is that decreased cell vulnerability is achieved at a metabolic cost to the cell; i.e., that there is

a "trade-off" between competitiveness for resources and resistance to infection. It is unknown if such a trade-off occurs *in vivo*. It is known, however, that the first step of infection of mammalian cells by many viral pathogens is binding to a cell surface receptor [9] and these receptors are often involved in important cellular functions. It is reasonable that the loss or modification of these receptors could result in a metabolic cost to the cell. If such a trade-off is not present, then the relationship between cell vulnerability and host nutrition predicted by our models may not occur. Our models also assume spatial homogeneity, a reasonable assumption for some *in vivo* environments at a small spatial scale, but an oversimplification for others. The presence of spatial heterogeneity can change the response of host cells to increased nutrients, as described above.

As we have demonstrated, the application of ecological theory to cell-pathogen interactions can result in new insights into within-host disease dynamics. Our research has shown that pathogen abundance can increase with increasing host nutrition, provided that invulnerable cells are not present. This is potentially a very important prediction, as pathogen abundance is an important determinant of the outcome of infection with many pathogens [15] and this prediction is worthy of further study in other cell-pathogen systems. The predictions of our models are not limited to predictions of average density, however. For example, these models also predict the effect of increased host resources on the stability of cell and pathogen populations and on the nature of cell and pathogen evolution [2-4]. These models can also be easily modified to include potentially important complexities such as multiple limitations on cell reproduction, spatial heterogeneity or the destruction of pathogens by the host immune system.

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