Defeating Drug Resistance in Pathogens: Guidance from Evolutionary Theory

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Abstract

Many of the greatest challenges in medicine and public health involve the evolution of drug resistance by pathogens. Recent advances in the theory of natural selection suggest that there are two broad classes of pathogen traits that can be targeted by drugs or vaccines. The first class, consisting of traits that benefit the individual organisms bearing them, causes a strong evolutionary response and the rapid emergence of drug resistance. The second class, consisting of traits that benefit groups of pathogen organisms including the individual provider, causes a weaker evolutionary response and less drug resistance. While most previous drug development has targeted the first class, it would be advantageous to focus on the second class as targets for drug and vaccine development. Specific examples and test cases are discussed.

Key words: multilevel selection; vaccine design; avian influenza; HIV; MRSA; malaria; tuberculosis

Introduction

Evolution by natural selection is the central organizing concept of biology. It is also increasingly recognized as being central to medical science (e.g., Williams & Nesse 1991, Trevathan et al 2007, Dethlefsen et al. 2008, Nesse & Stearns 2008). Many of the crucial features of pathogens, including traits that cause human illness and death, result from evolution by natural selection. Moreover, evolution is not only a process that has influenced the traits of pathogens in the past. Because microbes have very large population sizes and short generation times, they evolve very rapidly, so that evolutionary changes can be medically important on the time scale of a human life span or less.

Perhaps the most pressing medical problem resulting from pathogen evolution is the rapid emergence of drug resistance in many diseases. Microbial evolution within the body (somatic evolution) can be a major clinical issue in fast-evolving viruses (Mascolini & Richman 2008, Cannon et al. 2008), but also in bacterial infections such as tuberculosis (Li et al. 2005, Burman et al. 2006). Over a longer period, somatic evolution can drive the evolution of an entire multi-host population of pathogens (Wargo et al. 2007), leading to new or resurgent medical threats (Table 1). Evolved drug resistance in microbial pathogens has been described as, "the single greatest threat to the continued success of medical intervention" (Levin & Anderson 1999, p. 137), and it has been prominently suggested that we must prepare ourselves for the grim prospect of a "post-antimicrobial era" in medicine (Cohen 1992, p. 1050).

Table 1: Some current health threats involving drug resistance

Pathogen	References
HIV	Hogg *et al*. 2006
Malaria	Choi *et al*. 2008
MRSA (*Staphylococcus aureus*)	Klevens *et a*l. 2007
Tuberculosis	Anon. 2008, Zarocostas 2008
Avian influenza	Singer et al. 2007

Ongoing pathogen evolution is a crucial problem in medicine even beyond the domain of infectious disease. We are most accustomed to thinking about natural selection and evolution in a population of organisms, but the concept has wider applicability (Box 1). A population of reproducing cells can evolve drug resistance through natural selection even if they are not organisms, but instead cancer cells within an organism (Nowell 1976, Merlo et al. 2006, Pepper et al. 2007). Consequently, the development of drug resistance within a patient is a central problem in cancer biology (Moscow *et al*. 2003, O'Connor *et al*. 2007).

Advances in evolutionary theory are relevant to the problem of drug resistance. The basics of evolutionary theory have changed little since the writings of Charles Darwin. Despite its stable core, however, evolutionary biologists have continued to refine the theory and to extend it to account for more recent observations. Some of the extensions that are most relevant to medicine fall within the area of "multilevel selection" (Box 1). Historically, multilevel selection theory has been most important in understanding and predicting the evolution of social traits and interactions. The social interactions of microbes were long underappreciated, but are now recognized as being

both extensive (West et al. 2007a), and medically important (Foster 2005). To date, the primary application of multilevel selection in medicine has been in understanding the evolution of pathogen virulence (e.g., Bull 1994, Miralles *et al*. 1997)

Box 1: Principles of multilevel selection

Darwinian natural selection is an algorithmic process. It is independent of the specific substrate, and will operate on any population of reproducing entities if those entities have heritable variation in traits that influence reproductive success. Natural selection is most prominent and most often recognized among individual organisms, but the fundamental criteria for selection are also met at other levels of biological organization (Lewontin 1970). Recognition of this fact has led to a theory of multilevel selection, or selection at multiple levels of biological organization (Sober & Wilson 1998, Keller 1999, Okasha 2006). The idea of selection among groups and populations has been especially contentious, due to sweeping and poorly supported claims for the central role of selection among populations (Wynne–Edwards 1962), and negative reactions to these claims (e.g., Williams 1966). It is now generally recognized, however, that group-beneficial adaptations do arise through evolution, and that they are often best understood as resulting from selection among groups (Wilson 1997, Wilson & Wilson 2007). Little disagreement continues about the mechanisms of evolution, although some debate persists over terminology and semantics (e.g., West et al. 2007b).

For our current purposes, the crucial insights deriving from multilevel selection theory are these: (1) Unlike traits beneficial only to the individuals bearing them, group-beneficial traits are favored by selection among, and not within, groups. (2) Selection strictly among groups is weaker and slower than selection that operates both among and within groups. (3) The group–beneficial traits of microbes are often subject to conflicting selection within versus between groups, and consequently are typically less evolutionarily robust than are traits beneficial only to their bearers (MacLean 2008).

The proposal: target the group-beneficial traits of pathogens

Evolutionary theory thus tells us how to minimize the evolutionary response of a microbial pathogen when it is challenged with a new therapeutic agent. We cannot prevent natural selection and evolution from proceeding, but we can arrange for a slower and weaker response, so that our drugs retain more potency for a longer time. Because group-beneficial traits are typically created and maintained solely by selection among (and not within) groups, these traits should be the preferred targets for new drugs.

Among the well-understood group-beneficial traits of microbes, including pathogens, are many examples of "public goods". These are beneficial products produced by an individual organism that can then be used by the producer as well as its neighbors (West et al, 2007a). Pathogenic microbes secrete many public goods, including siderophores for scavenging scarce iron; extracellular matrix that protects against host immune cells and antibiotics; toxins with various effects; and quorum-sensing molecules that act as signals to coordinate the actions of multiple bacterial cells (Table 2). The relative weakness of natural selection for maintaining microbial production of public goods is highlighted by the observation that such traits are not uniformly maintained in

natural pathogen populations. For example, the longer an infection of *Pseudomonas* bacteria persists in a cystic fibrosis patient, the more likely the bacteria are to lose production of group-beneficial siderophore secretion (de Vos et al. 2001).

Table 2: Medically important group-beneficial traits of bacteria

Group-beneficial trait	References
Quorum-sensing molecules	Daniels et al. 2004
Siderophores	West & Buckling 2003
Secreted extra-cellular matrix	Davies & Geesey 1995
Secreted toxins	Dowling & Wilson 1998, Galan 2005

A mathematical model of the evolution of bacterial antibiotic resistance has supported the hypothesis that drugs targeting the group-beneficial traits of bacteria will be less vulnerable to evolved resistance than are antibiotics, due to smaller populations and reduced evolutionary rates associated with selection solely among groups, versus selection of individual cells both within and among groups (André & Godelle 2005). This insight can be extended beyond the context of antibiotic resistance to the broader problem of drug resistance, and the broader solution of targeting any group-beneficial trait of any pathogen. Some generally applicable theory can provide quantitative indicators of the potential benefit from this approach in any specific application (see Appendix).

Examples and test cases

Several group-beneficial traits of pathogens have already been adopted as drug targets, or suggested as such. Perhaps the most impressive success story to date comes from cancer medicine, which has been plagued by recurrent problems with acquired drug resistance. Oncology has a long history of searching for cytotoxic drugs that kill cancer cells. Unfortunately, such drugs act as powerful selective agents on the genetically heterogeneous populations of cancer cells found in most patients. Consequently, treatment with these drugs often causes tumor shrinkage followed by relapse and the resurgence of a new, drug-resistant version of the same cancer in the same patient. (Moscow et al. 2003). This pattern was broken when it was appreciated that tumors cannot grow and survive without triggering the growth of new blood vessels to supply them with oxygen and nutrients. Through random genetic changes, cancer cells accidentally discover how to express their genes for the angiogenesis factors that signal the vascular system and trigger the growth of new blood vessels. These angiogenesis factors, and the new blood supply they engender, are public goods initially provided by certain mutant cells that benefit all cancer cells in their vicinity, including themselves. When a patient is treated with an angiogenesis blocker, tumor growth soon leads to tumor starvation and suffocation so that disease progression is halted or reversed. Unlike the case of cytotoxic agents, tumors do not typically develop resistance to angiogenesis blockers (Kerbel 1991, Boehm *et al.* 1997). Partly because they retain their effectiveness and forestall relapse, these drugs have been one of the most important breakthroughs in the treatment of several types of cancer (Weinberg 2007).

Through the lens of multilevel selection theory, we can recognize in hindsight why it could have been anticipated that angiogenesis blockers would be less susceptible

than cytotoxins to evolved drug resistance. All genetic variation within a cancer originally arises in single cells. Imagine a cluster of cells within a tumor in which most cells produce an angiogenesis factor that can be blocked by a certain drug. Any mutation conferring resistance to this angiogenesis blocker must originate in one cell, allowing its angiogenesis factor to remain effective. A single cell cannot produce enough effective angiogenesis factor to attract the blood vessels it needs to survive, but a larger clone of descendants could. If a cytotoxin were applied, a single resistant cell would survive and recolonize the entire region. However, when an angiogenesis blocker is applied, most angiogenesis fails and all cancer cells in the region die, including any resistant cell producing effective angiogenesis factor in an insufficient amount. Although a larger clone of resistant cells could survive the drug and proliferate, it is unlikely that the original mutant cell could proliferate to create such a clone before the drug is applied, thus before resistance is advantageous. Instead, the lone resistant cells die as their neighbors die and take with them the public goods they had provided. Although not intended as such, the success of angiogenesis blockers in cancer treatment serves as a proof of concept for the idea of attacking public goods and other group-beneficial traits without triggering a strong evolutionary response and the rapid rise of drug resistance.

Parallel strategies have already been suggested for the development of new drugs against infectious pathogens such as bacteria. Many pathogenic bacteria cannot infect without relying on public goods such as quorum-sensing signals that allow multiple cells to coordinate their actions (de Kievit & Iglewski 2000). An individual cell that was resistant to a drug that blocks quorum-sensing could not coordinate with its drug-sensitive neighbors, and thus would not be positively selected. Therefore, Rassmussen & Givskov (2006) suggested that drugs blocking the effects of quorum-sensing molecules could protect against bacterial infection without provoking the rapid evolution of drug resistance that has been all too typical of antibiotics. This approach has also been advocated by other authors (Bergstrom & Feldgarden 2008)

Another proposed drug target for antibiotic-resistant bacteria is sortase, a cell-surface protein involved in the secretion of a variety of public goods by gram-positive pathogenic bacteria (including *Streptococcus,* lactococci, enterococci, and *Listeria*) Without sortase and the public goods it helps to secrete, most of these pathogens cannot sustain an infection (Cossart & Jonquières 2000, Maresso & Schneewind 2008).

As a final and more specific example of a potential application, the approach proposed here could address a problem that is currently a source of much concern. The bacterium *Streptococcus aureus* was formerly vanquished as a pathogen by the ready availability of effective antibiotics. By inadvertently selecting for the survival of any bacterial cells with greater resistance, antibiotics provoke a rapid evolutionary response. As a result, one by one our antibiotics have lost their effectiveness against many strains (Rowe-Magnus & Mazel 2006, Bergstrom & Feldgarden 2008). In particular, Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant cause of healthcare- and community-associated infections. Its prevalence continues to increase, and it is now a major source of mortality (Klevens et al. 2007). There have been many calls for the development of new antibiotics that are able to kill bacteria resistant to existing drugs, but it would be very optimistic to assume that the scenario that has played out so many times in the past will not be repeated after the introduction of yet another drug to kill bacterial cells. It is worth also considering the development of new drugs that will not

cause the rapid evolution of drug resistance. Potential targets for such drugs are abundant: *S. aureus* strains secrete more than fifty polypeptides, many of which are required for establishing infections (Sibbald *et al*. 2006). For example, α-toxin is a secreted protein toxin that digests host cells and tissues, turning them into available nutrients that support bacterial growth. As in the other cases discussed above, production of such a public good that is ineffective due to drug intervention does not select against a bacterial cell that can partake of the public goods provided by its neighbors. Thus if there is mutational variation among cells in their resistance to drugs that reduce the effectiveness of their secreted toxin, cells resistant to the drug will not survive or reproduce more than their neighbors and competitors, and thus will not come to dominate the population. *Streptococcus aureus* α-toxin is an excellent candidate target because it is required for virulence in several animal models (Bhakdi & Tranum-Jensen 1991), and is a well-characterized molecule (Tomita & Kamio 1991).

The strategy of targeting group-beneficial traits may not be applicable to every disease. However, because public goods are widespread in pathogens, there are many opportunities for applying this approach. For example, each of the cell phenotypes listed in Table 2 represents an opportunity for developing new anti-bacterial therapies that will not quickly lose effectiveness. Similarly, each of the traits listed in Table 3 represents an opportunity for developing new cancer therapies that will not quickly lose effectiveness. Some public goods produced by cancer cells are already known to be viable drug targets. For example, blocking the secretion of protease enzymes by tumor cells reduces invasion and metastasis in a mouse model, without reducing cell survival or proliferation (Farias *et al*. 1998).

Table 3: Medically important group-beneficial traits of cancer cells

Group-beneficial trait	References
Angiogenesis factors	Duda 2007
Secreted growth and invasion factors	Brünner & Dano 1993, Farias *et al*. 1998
Secreted immune suppression factors	Seliger 2005, Pries & Wollenberg 2006, Duthey et al. 2007, 2008

Discussion

Pathogens evolve resistance to the immune system, as well as to therapeutic drugs, in a process called "immune escape" (Read et al. 2004), or "antibody escape" (Frank 2002). This compromises the continuing efficacy of vaccines, and is an important problem in vaccine design (Althaus & De Boer 2008). The principles discussed here apply to the adaptive response of microbes to any challenge, whether pharmacological or immunological. Thus, group-beneficial traits of pathogens should be preferred targets for the design of vaccines as well as drugs.

The approach advocated here is untested in most applications. It can and should be further developed, both in more detailed theoretical models, and in preclinical and eventually clinical trials. In particular, key parameters are yet to be measured. The relative advantage of targeting group-beneficial versus cell-intrinsic traits can be quantified as a factor $g(1 + V_w/V_{tot})$, where g is the number of cell generations passed during the replacement of a cluster of cells with shared fitness effects, and V_w/V_{tot} is the

fraction of fitness variation among cells that falls into the within-cluster partition (see Appendix). The advantage of the proposed approach cannot be predicted quantitatively without an estimate of these key parameters, but it seems likely that $g \geq 1$ and $V_w > 0$, so that some advantage can reasonably be expected. Another caveat is that this approach predicts only robustness against evolved resistance. Drug efficacy is a separate consideration.

If it turns out to be useful, this strategy may seem obvious in hindsight. The fact that it is not already routinely applied may reflect past reluctance within the medical profession to fully embrace evolutionary thinking (MacCallum 2007).

The conclusions presented here do not depend on accepting the terminology or mathematical notation of group selection. It is necessary that we recognize the existence of group-beneficial traits in nature, but we have a choice of terminology and notation for explaining their evolutionary origins. Selection involving groups of kin can also be described and quantified using the concepts of kin selection and inclusive fitness. Partitioning selection into direct and indirect components is neither more nor less correct than partitioning it into within-group and between-group components. Indeed, it is well established that the two mathematical frameworks are formally equivalent, and inter-convertible (reviewed by Pepper & Smuts 2002). Because of residual aversion to the concept of group selection, some will prefer to avoid the language and notation of multilevel selection when one of the levels involved is any type of group. This is purely a matter of taste and convenience, and does not affect the conclusions. Whereas multilevel selection focuses attention on the partitioning of genetic variance into within- and between-group components, inclusive fitness theory focuses attention on the excess genetic similarity ("relatedness") of a cell to the rest of its cluster, relative to other cells. These are obviously closely related quantities that must be measured in similar ways. Public goods benefit the entire trait group (cluster) of recipients, including the producer. The quantitative analysis of such "whole-group traits" using an inclusive fitness framework was discussed by Pepper (2000).

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Appendix: Quantifying weaker selection on public goods.

Fitness variance within a generation

According to Fisher's 'fundamental theorem of natural selection", the rate of evolutionary change due to natural selection is directly proportional to the variance of fitness in the evolving population (Fisher 1930, Price 1972a). This rule also applies in the context of multilevel selection. For example, when selection occurs both among individuals within groups and among groups, the rate of change caused by selection at each level each is directly proportional to the fitness variance *at that level* (Price 1972b). It is therefore useful to partition total fitness variance into the sum of the within-group and between–group components: $V_{tot} = V_w + V_b$

Consider now the microbial trait of ability to survive drug treatment. Here the "groups" of interest are spatial clusters of cells that share the same micro-environment of diffusible substances, including public goods and drug-resistance factors. These clusters are an instance of what has been termed "trait groups", because the fitness of each cell in such a cluster is dependent upon the traits of the others (Wilson 1975, Pepper 2000).

For drugs that target an intrinsic property of the cell, drug resistance is also an intrinsic property of the individual cell. Some clusters or "trait groups" will have higher fitness than others, simply because they contain a higher proportion of resistant cells, so that fitness variance between groups (V_b) > 0. For clusters that are pure clones, variance within clusters (V_w) will be low due to common descent, while variance between clusters (V_b) is higher than it would be among non-clonal clusters. However, variance within clonal clusters (V_w) will be non-zero if there is any ongoing mutation within clonal lineages. As noted above, the rate at which resistance to such drugs evolves will be proportional to $V_{tot} = V_b + V_w$.

In contrast, for drugs that target public goods, drug resistance is a property not of the individual cell, but of its micro-environment of diffusible public goods. In this case, by definition, there is no resistance-related fitness variation within cell clusters. Either all cells within a cluster lose the benefit of their shared public goods, or through mutational evasion of the drug, all cells retain the benefit. Thus, $V_w = 0$ and the rate at which resistance evolves will be proportional to $V_{tot} = V_b$.

The consequence of this contrast between drug types is that the evolutionary change per cell generation will be greater for cell-intrinsic traits than for group-beneficial traits by a difference of V_w / V_{tot}. Evolution of resistance will be slower for drugs targeting group-beneficial versus cell-intrinsic traits whenever $V_w > 0$ for the cell-intrinsic trait, due to ongoing mutation within clonal lineages.

Effect of generation times

In addition to the amount of trait change per cell generation, we must also consider the number of generations per unit time as a factor influencing rate of evolutionary change. A drug that targets a cell-intrinsic trait will cause susceptible cells to die and be replaced once per cell generation. In contrast, a drug that targets a group-beneficial trait will cause susceptible cell clusters to die and be replaced once per cluster "generation". If a cluster develops through g generations of cell replication, g cell generations will pass during each cluster generation. All else being equal, this will result in a faster rate of

evolutionary change toward resistance for the drug targeting a cell-intrinsic trait by a factor of g.

Combined effects

Combining the effects of fitness variance and generation times, a drug targeting a group-beneficial trait will evolve resistance more slowly than will a drug targeting a cell-intrinsic trait by a factor of $g(1 + V_w/V_{tot})$, where g is the number of cell generations required to replace destroyed cell clusters., and V_w and V_{tot} denote the fitness variation within cell clusters, and within the total cell population, respectively.