Ecology and Medicines

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This year, more than 2 million people in the richest countries in the world will be overwhelmed by evolution and die. At the heart of that carnage is an ecological process that is barely being studied. My choice for Unsolved Problem in Ecology: what regulates the population densities of drug resistant pathogens, parasites and cancer cells?

The Grand Challenge

One of the greatest triumphs of 20th Century Medicine was the discovery of drugs that could be used to treat infections and cancer (1-3). But almost as soon as those wonder drugs were discovered, failures due to what we would now call drug resistance were observed. Every known cancer drug can fail for this reason, as can most antimicrobial drugs. Today, virtually all cancer deaths in rich countries are due to therapeutically resistant disease, where resistant populations of neoplastic cells come to so dominate a tumor that initially effective therapy no longer works (4). Likewise, drug resistant strains of microbes increasingly challenge global health in settings as diverse as American hospitals, Mumbai slums and animals food production systems. For cancers, resistance evolution plays out de novo in each patient. For infections, de novo evolution can be quite rare, but having emerged, drug resistant strains can rapidly spread globally. One estimate has it that by 2050, antimicrobial resistance will kill more people than cancer does today – if nothing is done (5).

Clearly, there is much that can be done. Inventing new drugs is important, but a constant search for the n^{th} generation drug to treat resistance to the $(n-1)^{th}$-generation drug (6) is not obviously sustainable, especially when resistance mechanisms get ever more generic and when it costs in excess of $US1 billion to bring a new drug to market. Non-drug solutions have to be a top priority – particularly ways to attack infections and tumors with biologics, like phage, vaccines and immunotherapy (although many of those approaches will also drive antagonistic evolution in the target cell populations). And of course for infections, apparently simple things like hygiene and alterations to farm practices can help. But for all that, it is hard to imagine that medicine can continue to deliver health gains in the 21^{st} century without heavy reliance on therapeutic chemotherapy. So we need to figure out how to use current and next generation drugs in a way that delivers therapy without also delivering drug resistance.

HIV treatment shows that is possible (7). The right combination of drugs delivered with the right regularity and at the right doses prevents the evolution of resistance to antiviral drugs and makes HIV

infection survivable. Apparently evolution-proof drug treatment therapies have also recently been developed for Hepatitis C virus (8). These strategies work by preventing resistance arising in the first place. If for example there is 10^{-8} chance that a mutation conferring resistance to a drug will occur, the chance that an individual pathogen or tumor cell will simultaneous acquire resistance to n drugs with different modes of action is 10^{-8n}, a vanishingly small number. But patients are not always fully compliant with the right regimens (7, 8), so periods of monotherapy can result. And even where patients can be relied on, combination therapy is not always possible. There can be limited drug options, especially because contrasting modes of action are required. Cross-resistance readily evolves anyway in many cancers and infections, and in the case of infections, de novo resistance can be a small part of the problem. For example, multidrug resistant TB is mostly caught from other people (9). Moreover, for many bacterial infections there is often good data that patient health outcomes are not improved by combination therapy (reviewed by Woods and Read (10)). Where immunity is going to clear an infection anyway, asking a patient to swallow the extra cost and side effects of an additional drug to perhaps prevent the spread of resistance in a hospital is tricky stuff.

So combination therapy can provide a solution to the problem of drug resistance but, at least as it is currently formulated, not a universal one. We have to explore other ways to use drugs to treat patients while minimizing the resistance evolution. I contend that there will be a myriad of solutions, but to get at them, we need to add some serious ecology to current efforts in oncology, molecular genetics, pharmacology and clinical microbiology.

The Ecological Challenge

Evolutionary rescue, the ability of a population under rapid decline to evolve traits to enable population recovery before extinction, is relatively well studied by evolutionary ecologists and geneticists not least in the context of climate change (11). An unfortunate phrase in the context of human health, evolutionary rescue is nonetheless precisely what is happening when drug resistance emerges in infections and cancer. And at the heart of it all, is ecology (12).

Figure 1 is the schematic used by the Centers for Disease Control and Prevention to explain to the public how antibiotic resistance evolves. The process is fundamentally the same for all the pathogens, parasites and cancers on which we wage chemical warfare. In the absence of drug treatment, the population size of resistant cells is tiny. After aggressive drug treatment, the sensitive cells are removed and the resistant cells replicate, sometimes to life-threatening densities (and, in the case of infection, oftentimes to transmissible densities). Thus, the sensitive population prevents the replication of resistance. Treatment removes the therapy-sensitive population, and a massive expansion of the resistant cell population ensues. It is that population which causes medical problems. This process can play out many times in a patient as treatment regimens are repeated or changed, but whatever the details, the key effect is the vast amplification of resistance. Before treatment, the sensitive population makes resistance so rare as to be of no concern (that is how we recognize a drug as being effective or useful in the first place). Afterwards, the ecology of the situation is so rearranged by chemotherapy that resistance has been amplified by many, many orders of magnitude.

Of course the resistance arises in the first place by some sort of genetic event such as mutation or, in the case of bacterial infections, from horizontal gene transfer from non-target organisms. Over the last few decades, science has generated vast catalogues of the genetic events that cause resistance in infections and tumors. But from the genetic details alone, we can infer rather little about what will happen once an individual pathogen or tumor cell has become resistant. Ecological forces determine its fate. We control those forces with our drugs. If we want to stop creating therapy-resistant cancers and pathogens, or deal with them once we have, we have to understand those ecological forces.

Drug resistance is thus a problem in applied ecology. When we want to do something endangered species, invasive species, pest species, infectious diseases... we need to first understand the ecological processes determining the dynamics of the populations of concern. Solutions come from that science. In agriculture, where problems of resistance to insecticides and herbicides are legend, many solutions have come from studying the ecology of the target organisms (13). The same must be true, surely, with drug resistant tumor cells, pathogens and parasites. Yet the ecology of resistance in patients is barely being studied. We often know in excruciating detail the genetic and cellular mechanics of drug action and resistance mechanisms. By contrast, our understanding of the ecology by which any resistance mechanism threatens patient health is rudimentary. I am not sure it is even yet at the level of Elton's *Animal Ecology* – published in 1927 (14).

Competition

Let me illustrate that claim. To me, the dominant ecological force which can account for the dynamics schematicized in Figure 1 is competition. Most obviously, competition with sensitive cells prevents resistance emerging once it has arisen. Competitive suppression explains why resistance is rare prior to treatment; competitive release accounts for the subsequent resistance explosion. Experiments bear out that interpretation. Figure 2 shows the ecological processes of competitive suppression and competitive release in play in experimental infections in my lab.

But, echoing earlier debates in ecology (15, 16), others do not think competition is important. For instance, Bruce Levin (quoted by Kupferschmidt (17)) says that because fitness costs of resistance are often not that high, competitive release is not a very strong force (see also 18). My view is that competition will still be important even if there are zero fitness costs to resistance, and that where experiments have been done (e.g. Fig 2), they clearly show competition. But without doubt, the importance of competition between drug-sensitive and drug-resistant cells in infections and tumors is an empirical question. A consensus cannot be reached until ecological experiments in a wide range of settings have been done. And incredibly, there isn't even consensus on how to do the relevant experiments. What are often called competition experiments in studies of antimicrobial resistance are often measures of relative growth rate in exponential growth phase, before any density dependence kicks in (19).

Even if competition turns out to be a key ecological force, as I strongly believe it will, that is only a starting point. What is the mechanism of any competition? Resource, interference or apparent (immune-mediated) competition (20)? My attempts to test for apparent competition in a mouse model of malaria, some of the only in vivo studies I am aware of, have proven frustratingly contradictory (21,

22). Quite possibly in that particular host-pathogen system, several types of competitive interaction are going on at once, with their relative importance changing during the course of infection. More generally, I know of no work looking at competition between resistance and sensitive lineages across resource gradients, the simplest and most fundamental ecological question. Are single nutrients limiting? Which ones? How does immunity modify that? Where is the density-dependence coming from? For infections, when is competition with drug-sensitive progenitors that is most important, and when is it competition with confecting strains or commensal members of the microbiome? Or is something else the reason we are not already neck deep in resistance?

The ecological processes controlling resistance in two arenas seem particularly important. With both tumors and bacterial biofilms, very strong competition must be going on; indeed necrosis is common within tumors as cells die from lack of oxygen and glucose (23). How do sensitive and resistance cells compete in those arenas? Is there competition on the growing edge of biofilms and tumors or are those regions so resource rich that there is no density dependence? Therapeutic drug concentrations can be very high on the outside of biofilms and tumors; how does that modify any competition? If we understood the ecology in those settings, I strongly believe we could make more informed decisions about dosing regimens, choice of drugs and drug combinations, and of non-chemotherapeutic solutions.

More generally, competition is quite possibly the only major natural force preventing the evolution of drug resistance. I have trouble imaging what else stops resistance emergence in the absence of treatment. Complex adaptive valleys that can't be crossed in the absence of drugs? That's competition. Drift? That's demographics. Waiting time for mutations? They seem to come along pretty fast when we use drugs. Whatever: some natural forces stops resistance spreading–otherwise we would not have anything we call a drug. What is that natural force? Can we harness it? Can we intensify it?

Coda

Every day, oncologists battle to keep their patients alive. When they lose that battle (as they will almost 600,000 times this year in the US alone), they lose it because chemotherapy has profoundly remodeled the ecology of a tumor. We know next to nothing about that ecology. It is of course easier to sequence cells than to measure growth rates while manipulating resource gradients, controlling immunity, hormones, cell-cell interactions and chemotherapy. But not so long ago, the idea of getting a complete DNA sequence for a single tumor cell was fantasy. I hope that in the not too distant future, we will be able to peer into a tumor or a biofilm, observe the relevant natural history and do decisive ecological experiments. I bet it will be hugely interesting. And save lives.

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Figure 1. Public health summary of the evolution of antibiotic resistance. Figure from Centers for Disease Control and Prevention http://www.cdc.gov/drugresistance/about.html

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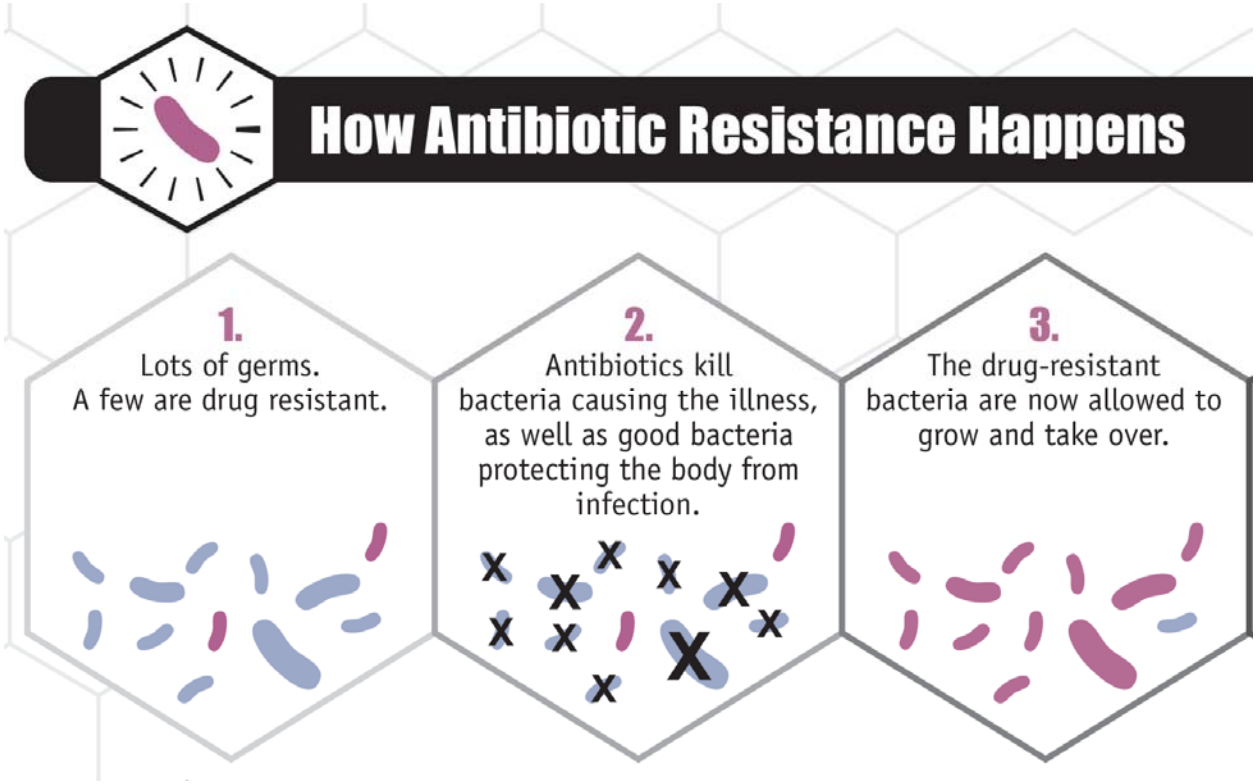

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Fig 2. Competition in a mouse model of malaria. Kinetics of infections in nine mice infected with pyrimethamine-resistant (red) and -sensitive parasites (black). Mice were infected with 25 resistant parasites (red dots); some mice were infected five days earlier with a million sensitive parasites (black dots). Mice G-I were treated with pyrimethamine for seven days (gray bars) to eliminate the sensitive parasites. Note that, formally, red flat lines denote teimes at which densities were below PCR detection. Note that otherwise proliferating populations of resistant parasites (Mice A-C) are competitively suppressed by sensitive parasites (Mice D-F). Drug treatment releases them from competitive suppression (Mice G-I). Figure from (24) (reproduced with permission*).

*not yet obtained...