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The Acquired Immune System: A Vantage from Beneath

Commentary

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The immunity exhibited by plants and animals is often viewed as the evolutionary response to the problem of infectious agents. In this respect, the combination of the innate immune system and the acquired immune system has been characterized as the "optimal solution." In this essay, I propose that there is no possibility of an optimal solution to the problem of parasitism. Regardless of the immunological mechanisms evolved, infectious agents establish a dynamic interaction with common strains of their host species, weighing virulence against transmissibility. In the endless host-parasite coevolution, the immune system can never gain an upper hand on the millions of parasitic microbes and viruses. Rather, evolution of the immune system is driven, most importantly, by the small advantages conferred as a result of host variation. By selecting for ever-more-devious parasites, the immune system is the cause of its own necessity.

Introduction

Immunologists, myself included, have long thought about the immune system as if it were of crucial importance in defense against infection. The thinking is that the "immune-system genes must evolve to keep pace with increasingly sophisticated evasion by pathogens" (Trowsdale and Parham, 2004). The acquired immune system, signaled into action by the innate immune system, is seen as an optimal host defense (Janeway and Medzhitov, 2002). The proof of this is thought to be that most infections are cleared (Trowsdale and Parham, 2004). I think this is a perspective that could benefit from broader evolutionary point of view. If the vertebrate immune system has evolved to provide "optimal host defense," then an implication is that invertebrates, lacking an acquired immune system, should be rife with pathogens and frequently succumb to infections. If the vertebrate immune system has evolved to keep pace with increasingly sophisticated mechanisms of pathogenesis, why are diseases such as cholera, measles, malaria, ancylostomiasis (hookworm), and leishmaniasis endemic in much of the world? If it is an evolutionary solution to infectious disease, why did influenza kill 40 million people in 1918? In fact, is there any evidence that vertebrates experience less morbidity and mortality due to infectious disease than invertebrates? The solution is to realize that we have an anthropocentric perspective. We (and most military planners) forget that the targeted enemy has a life or death stake in avoiding our strategies for defense. In fact, parasitic agents (meaning infectious bacteria, fungi, parasitic invertebrates, and viruses) only exist if they've managed to avoid their host's immune system, at least long enough to replicate and send their next generation on to a new host. No infectious agent is descended from an ancestor that was killed before it could replicate. In fact some parasitic agents can have geologically long relationships with their host species such that the two are really coevolved. Despite the evolution of a multifaceted immune system, parasitism is a fundamental principle of life.

Animals represent a wonderfully rich habitat including almost limitless energy and a stable environment for replication. Thousands of microbial agents and viruses have evolved to carve out parasitic niches; in fact, there are far more obligate parasitic species than free-living species of plants and animals (Price, 1980). If we think of a food chain or food web in terms of large organisms eating smaller ones, then the relationship between parasites and their hosts can be thought of as an inverse food web. Small organisms develop the ability to tap into the resources of larger ones—eating them from the inside out. This is a true web of interactions since vertebrates often harbor multiple parasites, the invertebrate parasites have parasites, and the parasites' parasites have parasites.

A constraint on a parasite's strategy is that the potential host can have a strong motivation to avoid being parasitized. It can mean loss of reproductive fitness. On the other hand, an obligate parasite is under an even stronger selective pressure. It must find a host, or its lineage is history. Furthermore, a parasite can be of any biological form, from a complex animal to a virus, and its generation time is short. Bacteria can undergo 100,000 generations for each one of ours. No matter what "defense" a host can muster, there will always be a parasitic agent that can avoid it to achieve replication and transmission. A potential constraint on a parasitic agent is that it can avoid many immune strategies, but not all simultaneously. The cost is too high. For example, carrying episomal antibiotic resistance genes is a burden for the bacterium since it is more costly to replicate. In the absence of antibiotic selection, despite plasmid-based mechanisms to promote retention, resistance is eventually lost (Bingle and Thomas, 2001). Success in parasite replication depends upon overcoming the selective pressures brought to bear by the host while carrying along a minimum of extraneous defensive machinery; however, reproductive success is more than just producing the most progeny—it is producing the most progeny who can themselves produce the most progeny. It is producing the most progeny that can successfully find a new host in which to replicate.

The host-parasite relationship is intimate. If parasites are successful in infecting a certain host strain, that strain may be scarce in the next generation. Thus, in the next generation, the most successful parasites would be those variants that can infect a different strain. Likewise, in any given generation the most successful host, able to ward off infections, represents the largest target for the next generation of parasites (Hamilton et al., 1990; Thompson, 1994). Leigh Van Valen proposed this type of frequency-dependent selection and coevolution as a new evolutionary law, The Red Queen Hypothesis (Van Valen, 1973). He cited Lewis Carroll's Red Queen, "Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that." In this context there is never a "solution" to infectious agents.

Even the acquired immune system, with its boundless plasticity, did not get us "somewhere else." Each solution instead has within it the seeds of its own demise.

The Enigma of Invertebrate Immunity

Plants and the vast majority of animals on earth have no acquired immune system; rather, they have a multiplicity of mechanisms to prevent infection that we collectively term innate immunity. I wish to emphasize that the most effective innate mechanism is the denial of access. Without barriers to infection, there are no possible cell and molecular devices that would be able to prevent rampant parasitism. In addition to barriers, the innate immune system is based on a set of rules that translate into a proscription against the display of pathogen-associated molecular patterns (PAMPs) not present within free-living multicellular organisms. These rules have evolved over hundreds of millions of years, they are passed on intact to each new generation, and they are manifest in the specificity found in receptors and mediators of the innate immune system: Toll-related receptors, mannose receptors, defensins, complement, peptidoglycan recognition proteins, the coagulation reaction, and many others, some of which have yet to be discovered (Cooper et al., 1992; Vilmos and Kurucz, 1998; Soderhall and Cerenius, 1998; Hoffmann and Reichhart, 2002; Janeway and Medzhitov, 2002; Steinert et al., 2003; Rolff and Siva-Jothy, 2003; Dziarski, 2004; Theopold et al., 2004). Cells respond to PAMPs by setting in motion a number of physiological changes designed to slow microbial growth or viral replication. Specialized cells, such as hemocytes, can be called into the fray. We think of the innate immune system as having been selected to prevent the initiation of an infection and limit the replication of infectious agents. In principle, it should exhibit little memory, with each incident of infection treated as a surprise; however, there is evidence that immunization of invertebrates can be protective (Keith et al., 1992; Muta and Iwanaga, 1996; Kurtz and Franz, 2003).

The invention of an acquired immune system at the dawn of vertebrate evolution was the raw material for rapid variation and selection. Whereas the innate specificity for pathogens must have evolved by trial and error at each generation, the acquired immune system could be selected to provide vast potential for recognition. The problem of refining each specific receptor on an evolutionary time scale was eliminated. As its name implies, the specificity for pathogens is acquired anew in each individual. This novel mechanism of selection must, at one time, have conveyed a strong advantage since all vertebrates, other than jawless fish, appear to have descended from a single species, a species that evolved exactly three lymphocyte types, each expressing a unique antigen-specific, clonally distributed class of receptor: $\alpha\beta$ T cells that recognize antigen peptides presented by MHC molecules, antibody-producing B cells, and $\gamma\delta$ T cells possibly necessary for negative regulation (Rast et al., 1997; Nam et al., 2003; Hayday and Tigelaar, 2003). The antigen receptor genes all require gene rearrangements mediated by orthologs of the recombination activating genes (RAG) and utilize terminal deoxynucleotidyl transferase for diversity (Miracle et al., 2001; Bartl et al., 2003). Over millions of years, this ancestral vertebrate species evolved the process into a complex physiological system with the basic components of the present day acquired immune system. A conclusion is that from sharks to aardvarks (as well as velociraptors and pterodactyls), all vertebrates arose from this aquatic predecessor lineage that happened upon the process of gene rearrangements.

Exactly how does the acquired immune system convey a selective advantage? Since an acquired immune response requires days to become effective, it is mainly directed toward combating an infectious agent that has gained purchase despite barriers and other innate mechanisms of immunity. Studies on a number of bacterial pathogens have shown that the acquired immune system is important in resolving an infection that is initially controlled by innate immunity (Nauciel, 1990; Weintraub et al., 1997). It also confers memory upon surviving individuals, such that reinfection is much less likely.

A novel type of immune system has been recently revealed to exist in our most distant vertebrate relatives, the jawless fish (Pancer et al., 2004). Lampreys presumably branched off the vertebrate lineage prior to the invention of RAG-mediated gene rearrangements, and they have apparently developed a parallel alternative to the acquired immune system. This immune mechanism is based on clonally distributed leucine-rich repeat receptors (similar to Toll-like receptors) that appear to be diversified by a novel mechanism of genetic rearrangement. It may be focused on the recognition of PAMPs, or it may be able to combine leucine-rich repeat domains to extend the recognition specificity to a larger set of biochemical determinates. The distinction has important implications for self versus non-self recognition in these animals, but the possibility exists that the lamprey immune system has selective aspects of both acquired and innate immunity. If it is restricted to the recognition of PAMPs, then it lacks the attendant costs associated with self-recognition (see below), and yet the organism may still benefit from the immune memory associated with clonal expansion. Such an immune system seems to blur the distinction between innate and acquired immunity.

Of the many implications from this discovery, one is yet another affirmation of the notion that evolution is not directed toward an optimal solution. There are many biological solutions to each problem, and that which is selected is greatly influenced by chance. Since a clonally selected, somatically diversified receptor system evolved at least twice, I conclude that it most certainly conveys a selective advantage in a world of infectious agents.

Why, then, is innate immunity sufficient for the most abundant species on earth, but not for vertebrates? After all, even a minor congenital deficiency in vertebrate acquired immunity is often incompatible with life. It's rarely discussed, but one idea is that long-lived, complex vertebrates require an acquired immune system (Janeway and Medzhitov, 2002). The implication is that invertebrates are simple and have short generation times relative to most vertebrates, and perhaps they can afford a high casualty rate. In case these ideas are attractive, we need to consider the existence and success of large and complex invertebrates, such as giant squids, clams, tubeworms, lobsters, oysters, sea urchins, or even insects. To take the argument to the extreme, we might consider plants, since they too have a parasitic burden.

The giant sequoia can live 2000 years and the ancient bristlecone pine can live past 4000 years. If acquired immunity is defined by somatic diversification and clonal selection, then as far as we know, none of these species appears to require an acquired immune system to avoid deleterious infections.

Clearly, the explanation is quite different, and if nothing else, I hope that this article provokes immunologists to consider in detail the evolutionary significance of the acquired immune system. I propose that we are looking backward from a human perspective and perhaps asking the wrong question. Perhaps the question is not why invertebrates manage to succeed in the absence of an acquired immune system, but rather, why do we vertebrates have pathogens that necessitate acquired immunity?

Virulence Theory and Immunity

The role of the immune system in vertebrate versus invertebrate evolution cannot be understood in the absence of virulence theory. One way to define virulence is the utilization of host resources by the parasite with the attendant costs to the host in terms of morbidity and mortality. At the two extremes, commensal organisms coexist with their hosts in a completely benign or mutually beneficial manner, whereas parasites utilize host resources, immobilize the host, and cause death in a high percentage of infections. Many fall somewhere between, exacting a price in terms of host resources without impeding host mobility. The differences seem to be tightly interwoven with mode of transmission or, alternatively, the ability to infect multiple hosts (Read, 1994; Cooper et al., 2002; Ewald, 1995; Day, 2003). For parasites that are directly and exclusively transmitted from one vertebrate to another, virulence appears to be calibrated such that the host retains mobility. Too virulent, and the parasite immobilizes or even kills the host before its progeny can be passed on. Too benign, and it is out competed by faster growing variants. An important point is that high virulence is dominant. The entire population of parasites within a host pays the price of a highly virulent variant.

How can this be reconciled with the existence of parasites that are extremely virulent? High virulence is strongly correlated with parasitic agents that can effect transmission other than by direct contact between hosts (Ewald, 1999). One effective mechanism is to utilize an intermediate vector. Agents such as flaviviruses (causing diseases like dengue fever, yellow fever, or West Nile fever) or *Plasmodium falciparum* (malaria) maximize replication while paying less of price for host incapacitation. In fact, an infected host lying immobilized but alive is even more susceptible to the bite of a mosquito, and the mosquito thus acts an agent for the parasite. A second mode of infection for highly virulent agents is water transmission. Microbes, such as *Vibrio cholera*, cause terrestrial animals to excrete copious quantities of infectious fluids, and without extraordinary precautions, they are transmitted through the water supply. Of course, the parasites of marine organisms are readily transmitted through water. A third strategy is for an agent to be highly enduring. Bacilli such as *Bacillus anthracis* can form spores that lie in wait for years, even under extreme conditions, and thus become transmitted through the mobility of healthy potential hosts. Predictably, once infected with *B. anthracis*, the acquired immune system offers the host little protection.

Another example of high virulence can occur when an infectious agent can not only replicate in one species without extracting a high cost (low virulence), but also infect a second species where it is highly virulent. For example, Ebola virus kills a large percentage of infected patients, great apes, and monkeys, but epidemics are local and appear to expire quickly. Since it continues to crop up, we assume that it has been selected to propagate in a natural host (as yet unknown) in which it is less virulent (Leroy et al., 2004). The high virulence in humans may be, in a sense, accidental. Influenza infects wild birds without causing obvious pathology, whereas it can be highly virulent in human beings (Hilleman, 2002).

This is an oversimplification of the complexity of virulence (Day and Proulx, 2004), but it is clear that no matter what the mode of transmission, there are still costs to the parasite associated with virulence. This does not mean that parasites naturally evolve to a benign state, but no parasite would be successful it if ravaged a host before it could be transmitted.

How do the principles of virulence help to explain the enigma of invertebrate immunity? One answer is that invertebrates don't need an acquired immune system because they never had it. The parasitic agents of invertebrates have not coevolved with acquired immunity so their virulence is calibrated to the coevolved innate immune system. The proposal here is that contrary to widely held views of practicing immunologists, the immune system is not evolutionarily selected to prevent infection in an absolute sense. Rather, it is selected to make one individual slightly more resistant or at least different than others of the same or related species. The adversary of any individual is not really the world of parasites, they are truly undefeatable, it is his or her neighbor. A zebra doesn't have to outrun the lion, just the slowest member of the herd.

Another way of looking at this is that acquired immunity was not a final solution to the problem of parasitism. There is no final solution. As novel as the acquired immune was, for rapidly multiplying agents, it was just another hurdle. It may have driven parasites to invent new strategies for fitness, but it did not convey invincibility or anything like it. To say the combination of innate and acquired immunity is the optimal defense is a misunderstanding of the evolutionary landscape. I don't believe there is an optimal defense. I don't believe there is a conceivable immune system that could not be obviated once the barriers to infection have been breached. For all animals and their parasites, generation upon generation, it has been evolutionary thrust and parry, until today as it was a million years ago and as it will be a million years hence, each and every species is literally plagued by parasitic microbial agents and viruses.

Secondarily, there are multiple factors that may affect the evolution of different forms of defense in species that are physiologically and ecologically disparate. There is a high cost to developing and utilizing even the innate immune system (Moret and Schmid-Hempel, 2000), and the adaptive immune system, with its surfeit of cellular production, is likely to be even more resource intensive. This does not explain how most animals are successful

Figure 1. Evolutionary Alice Through the Looking Glass

"The most curious part of the thing was, that the trees and the other things round them never changed their places at all: however fast they went, they never seemed to pass anything. 'I wonder if all the things move along with us?' thought poor puzzled Alice." (Carroll, 1872). Originally applied by Van Valen to frequency-dependent coevolution (Van Valen, 1973). In this case, Alice is a metaphor for animals unable to put distance between themselves and their ever-present parasites. Illustration by Kristina Hedrick, Lightray Productions, Los Angeles, California.

without an adaptive immune system, but it argues that invertebrates could probably not afford the energy expenditure it would require. The field of ecological immunology has emerged to study just this problem (Rolff and Siva-Jothy, 2003). The problem for biologists is to understand how substantially different strategies of defense can be equally successful in host-parasite evolution.

But We Seem to Be Protected?

The immune system was not evolved to protect us? This seems counterintuitive. We see that the immune system is absolutely essential to survival in a world of infectious agents, and we conclude that it was selected to prevent disease. The problem is it doesn't prevent disease. Once infected, are we really protected from influenza, tuberculosis, coccidioidomycosis, or toxoplasmosis? In the match-up between host immunity and parasitic selection, there's no contest. Like Alice pacing the Red Queen, we never get anywhere (evolutionarily) even though we continue to run as fast as we can (Figure 1).

Once the acquired immune response was invented, of course, there was no going back. Any individual with a defective acquired response would be quickly eliminated by a parasite expecting a full armament. Even commensal flora could become pathogenic. Regardless that infection of such a host might be a dead end for the parasite, an immune compromised individual would immediately succumb to a pathogen that would appear overly virulent. Moreover, pushed by parasitic selection, the acquired immune system has continued to find novel ways of conferring host advantage; not host immunity, host advantage.

The Success of Invertebrates

If the proposal is that the outcome of parasitism is predominantly determined by the parasite and not by the intricacy, strength, or elasticity of the immune system, then, when compared with vertebrates, invertebrates in their native ecosystem should not exhibit a mortality rate that is predominantly determined by infection and pathogenesis. Free-living invertebrates and their parasites should exhibit the same types of relationships that we find for vertebrates—a dynamic interaction in which parasites weigh the use of resources (virulence) against transmission. There should be benign parasites that minimally affect host behavior as well as highly virulent parasites that utilize multiple hosts or exhibit other characteristics that ensure their transmission. The longevity of invertebrates in the wild will undoubtedly be influenced by infectious agents, as it is in vertebrates, but a prediction is that it is not primarily limited by the absence of an acquired immune system. Instead, it should be more importantly tied to other factors that have been found to affect aging and survival. To make this point, I wish to briefly address four aspects of invertebrate biology related to survival and parasitism: the lifespan of invertebrates, the causes of death in insects, parasites that infect both vertebrates and invertebrates, and insect viruses.

Lifespan. As already noted above, the first issue may be addressed by considering the observed life span of invertebrates, and there is no doubt that complex invertebrates can be extremely long lived. There exist representations from the phyla of arthropods (lobsters, spiders, insects) (Ennis et al., 1986), mollusks (clams, squid) (Ropes, 1999; Cargnelli et al., 1999), and echinoderms (sea urchins) (Ebert and Southon, 2003) with life spans as long or longer than vertebrates. For example, red sea urchins (*Strongylocentrotus franciscanus*) and ocean quahogs (*Arctica islandica*) can live to be more than 200 years of age. Lobsters (*Homarus Americanus*) are known to live to be at least 30 years of age (Herrick, 1977; Campbell, 1983). So at least some members of invertebrate species live for decades in the wild without the advantages of acquired immunity; however, an important question concerns average life expectancy. Are 30-year-old lobsters the rare individuals who, by chance,

escaped infection? Did the occasional Quahog manage to live past 100 years whereas most of its kin succumbed to disease? This is not the case for many species that have been studied, and it does not fit with the current understanding of longevity. The idea is that the evolution of longevity is highly influenced by extrinsic mortality. As a majority of the population is lost due to predation or infection over time, there is a progressive weakening of the force of selection. The result of this is that the rate of aging is inversely correlated with the average lifespan in nature (Kirkwood and Austad, 2000). The converse is also likely to be true. Animals that can be found to be long lived must come from a population with a long average lifespan.

More convincingly, the analyses of invertebrate life tables show that mortality rate is not necessarily different in vertebrates and invertebrates (Carey, 2001). This is a vast topic that I can't consider in detail here, but I'd like to list two examples. A species of subsocial dung beetles (*Passalidae)* has an average lifespan of greater than 2 years in the wild (hardly a clean environment!), and approximately 5 years in captivity (Cambefort and Hanski, 1991). This is not so different from our favorite species for studying acquired immunity, the house mouse, *mus musculus*, which has an average lifespan in the wild of approximately 1 year and a lifespan in captivity of 2–5 years. A second example is the lobster (*Homarus americanus*), which has been studied extensively due to its commercial importance. Lobsters reach sexual maturity at 5-8 years. Including predation, disease, and storm damage, the natural mortality rate of juveniles and adults (excluding human harvesting) is very low with estimates ranging from 2%–8% per year (Thomas, 1973; Ennis et al., 1986; Fogarty, 1995). Adult lobsters in the wild are relatively free of disease (pathogenic protozoans, fungi, and metazoan parasites) (Fisher et al., 1978), as many diners can attest, although bacterial and parasitic infections have been detected when lobsters are subjected to suboptimal culture conditions, e.g., poor water quality, low oxygen tension, or overcrowding. In particular, a virulent form of *Aerococcus viridans* has caused high mortality in high-density holding conditions, but it has not been found in wild populations (Stewart, 1980). In neither example, nor in many others, does there appear to be a dramatically high casualty rate in mature invertebrates from infectious agents. I note that this excludes the larval stage of many invertebrates where the brood size and mortality rate can both be huge. Although beyond the scope of the present analysis, this reproductive strategy is often correlated with high levels of predation.

Another way of understanding the causes of death without knowing a detailed life history is to compare the life spans of related species living in similar conditions, and for social insects, to compare different castes within the same species. The range of lifespans in insect species is enormous—some live a few days, whereas others live years (Wilson, 1971; Carey, 2001). In addition, lifespan can also vary within a species depending on social status. Especially for subterreanean ants and termites, the workers live weeks, whereas the queens can live up to 30 years (Bourke and Franks, 1995). All individuals come from the same breeding stock, they live in the same environment, they are exposed to one another, and yet longevity is vastly different. Factors that determine extended longevity in insects are rather parental care, monogamy, and eusociality (Carey, 2001). The likelihood is that longevity is not primarily determined by environmental factors such as pestilence, but rather it is programmed.

Causes of Death. The most direct method to address the role of infection in invertebrate lifespan is to determine the proximal causes of invertebrate mortality. For insects these include predators, parsitoid insects, and nematodes, and infectious agents including fungi, protists, bacteria, and viruses. An analysis of the published life tables for 78 herbivorous insect species was carried out (Hawkins et al., 1997), and death was classified by enemy type: parasitoids; predators; and pathogens. A conclusion of the study was that herbivores examined through the pupa stage suffer little or no mortality from pathogens. Considering that sick individuals may be more susceptible to predation, even the combination of infectious agents and predation contributed relatively little to mortality. The overwhelming cause of mortality was found to be due to parasitoid insects and nematodes. Is this mortality due to the lack of an acquired immune system? Realizing that most parasitoid species do not invade adults and embryos would not be expected to have an organized immune system, this seems unlikely. In addition, the acquired immune system is notoriously poor in ridding the body of parasitic nematodes. The authors conclude that, "on average it [mortality from pathogens] does not represent a potent mortality source in phytophagous insect populations"(Hawkins et al., 1997). While the number of invertebrate species is vast and while some species may well be plagued by infectious agents, this does not appear to be a consistent characteristic associated with a lack of acquired immunity.

Versatile Infectious Agents. How does infection, such as viral infection, affect vertebrates versus invertebrates? One interesting example comes from the flaviviruses that need to replicate in both invertebrate and vertebrate hosts (Gould et al., 2003). They are the etiological agents of dengue fever, yellow fever, Japanese encephalitis, tick-born encephalitis, and the West Nile encephalitis. Control of infection by the host immune system requires type I and type II interferons and their induced effector molecules, as well as components of complement. Evidence also seems to support a role for the acquired immune system both in immunity and pathology (Kurane and Ennis, 1992; Mullbacher and Lobigs, 1995; Gagnon et al., 1999; Mongkolsapaya et al., 2003; King and Kesson, 2003; Wang et al., 2003). Some of these viral infections cause a high mortality rate in humans (yellow fever, $>$20%), whereas others are often, but not always, cleared with little associated pathology (West Nile Virus). Those neurotropic viruses that are not cleared end up in the brain, and this might be benign were it not for the immune response to this invasion resulting in lethal encephalitis. On the other hand, the arthropod vectors appear to maintain a lifelong infection that otherwise has no known effect. As such, the invertebrate host does not clear the infection but experiences little pathology, whereas the vertebrate host sometimes clears the infection, but when it fails,

the ensuing host-parasite conflagration can be dire to the host.

I think there are two lessons learned from these viruses. One is that regardless of the type of host immunity, a virus is able to achieve its ends of replication and transmission. The second is that an acquired immune system is not necessary to prevent a viral infection from causing death; rather, the lethality of a virus may more importantly depend upon its strategy for replication and transmission. In this case the virus most probably gains wider transmission by utilizing fewer resources of the vector and greater resources of the vertebrate host.

The life cycle of trypansomes, such as *Trypanosoma brucei*, provides another illustration of the way in which a parasite can adapt to the immune systems of both invertebrate and a vertebrate hosts. Trypanosomes are most famous for their ability to express a single variant surface glycoprotein (VSG) and then switch to a completely different VSG at a rate of 10^{-2} to 10^{-7} switches per doubling time (Turner and Barry, 1989). Importantly, this only occurs during the life-cycle phase in which the trypanosome is in the mammalian bloodstream and subject to antibody-mediated inhibition; high-frequency antigenic variation does not occur in the tsetse fly (Donelson, 2003). Although trypanosomes are most famous for antigenic variation, similar strategies are used by other parasites, such as *Plasmodium falciparum* and *Giardia lamblia*, during the mammalian phase of their life cycle (Nash, 2002; Duffy et al., 2003). Antigenic variation is also widespread in bacterial pathogens and especially in their virulence factors (van der Woude and Baumler, 2004). Much of this vast biological invention is almost certainly in response to the acquired immune system of vertebrates, and it consistent with the notion of adaptive virulence.

Insect-Virus Interactions. The interactions between insects and their specific viruses are endlessly complex, though field studies have established some important general characteristics that illustrate the diversity of pathogen interactions with invertebrate hosts. For example, the invertebrate iridescent viruses (IIV) are known to infect 73 invertebrate species, mainly insects. In the black fly *Simulium variegatum* found in Wales, inapparent IIV infections were found at frequencies between 0%–37%, depending on the time of the year (Williams, 1995). Transference of the flies to the laboratory confirmed that the infections were inapparent and nonlethal. The same group analyzed *Simulium spp*. from Chiapas, Mexico, and found patent IIV infections in eight *Simulium* species. This virus (or viruses) was distinct from that found in Wales in that it would not infect a test strain of moth, *Galleria mellonella*. The patent infections were uniformly associated with death before metamorphosis. Interestingly, the frequency of infection was directly correlated with species proportion (Hernandez et al., 2000), a hallmark of frequency-dependent coevolution. Clearly, depending upon the virus and the host, infections can vary in frequency and in virulence. For an introduction into this realm of study see "The Insect Viruses" (Miller and Ball, 1998).

These examples illustrate that acquired immunity is not a prerequisite for survival in the face of infections, nor for long average lifespan in complex animals. It may be an incremental advantage, but the answer to why most animals don't require an acquired immune system must lie elsewhere. I submit that a key lies in the strategies of parasitism.

A Moment of Evolutionary Ecstasy Bought Us 400 Million Years of Misery

Evolution has no foresight. A biological invention that confers an advantage gets propagated whether or not it may eventually lead to trouble or even species extinction. If the vertebrate acquired immune system is not necessary for evolutionary survival, we might even be so bold as to ask whether it was an evolutionary misstep. As discussed above, it must have provided a potent selective advantage, and it may even have contributed importantly to the success and rapid diversification of vertebrates. However, it also came with attendant costs. By inventing a weapon able to recognize any possible biochemical determinant and thus potentially destroy any cell, be it friend or foe, vertebrates developed the potential for self-destruction for the first time in biological history. The acquired immune system also placed a strong and unpredictable selective pressure on parasites, one that would be expected to provoke a reaction.

The reaction of viruses, seemingly the simplest of parasitic agents, has been recorded in their genomes, which collectively encode decoys and diversions designed to target each step in the process of immune recognition, activation, and function (Smith and Kotwal, 2002; Koelle and Corey, 2003). Moreover, viruses and microbial agents are likely to invent strategies that go beyond immune avoidance. They are also likely to subvert the host immune system as a means of furthering their ends of replication and transmission. For example, bacterial enterotoxins cause an immune holocaust that results in the excretion of copious quantities of infectious fluids. Retroviral gene products cause the activation and cell division of a large percentage of T cells, a requirement for viral replication. Because immunopathology is perhaps the most common adverse outcome of viral infections, I suspect there are thousands of viral and microbial strategies aimed at subverting the acquired immune system. As an aside, I think a comparison of vertebrate and invertebrate viruses would be revealing, and an obvious prediction is that viruses that exclusively infect invertebrates are simpler and focused on evading innate immunity.

The immune system of modern vertebrates is a coordination of innate immunity and acquired immunity. A response is often instigated by the innate immune system, which in turn activates components of the acquired immune system. The acquired response feeds back by amplifying the innate immune response, arming it with specific receptors and inducing potently lethal mediators. It also feeds back on itself via cytokines and other mediators. Such a scheme should worry any systems analyst. A potentially lethal mechanism controlled by positive feedback is a recipe for runaway destruction. Not surprisingly, we find that several hundred million years of evolution has mitigated that danger with preventative and negative feedback controls. We weed out self-reactive lymphocytes in the thymus or the bone marrow or the bursa. In the absence of innate activation, antigen-stimulated lymphocytes are poorly activated or actively introverted. Successfully activated lymphocytes are temporally programmed to self-destruct. Reg-

ulatory T cells are purposed to quell any conflict. Still, with all of these mechanisms in place, the evidence shows that negative regulation quite often fails or it is subverted by self-serving infectious agents.

So being in possession of an acquired immune system, vertebrates pay a high price in terms of immunopathology, but that is hardly the only cost. Immune hypersensitivity, including allergy and asthma, affects a large segment of the human population in the Western World. And of course the most dreaded of reactions mediated by the acquired immune system is *horror autoxicus* (Erlich, 1906), commonly known as autoimmunity. More than 3% of people in the United States experience a form of autoimmune disease (Jacobson et al., 1997; Cooper and Stroehla, 2003) that can be debilitating or even life threatening. This is not limited to humans, although we are the most comprehensively studied species. Several strains of mouse and rat have been found to have a high incidence of autoimmune disease, and this may represent an exaggeration of traits that exist in naturally breeding populations. Domestic dogs have been found to have many different autoimmune diseases such as rheumatoid arthritis, lupus, and diseases of the skin (Fleeman and Rand, 2001; Hansson, 1999; Olivry and Jackson, 2001). On the other hand, excepting cancer, who ever heard of neutrophils raging out of control? Where is there evidence that flies can be struck down by overzealous hemocytes?

Was the acquired immune system an evolutionary misstep? It is pure speculation, but I believe that vertebrates would have evolved quite differently or even not at all had the progenitor lineage not happened upon RAG-mediated gene rearrangements or at least some method of generating a somatically diversified, clonally expressed recognition system. Over hundreds of millions of years of host-parasite interactions, even an incremental advantage would be expected to completely alter the evolutionary outcome. So to say that the acquired immune system is an evolutionary mistake is nonsensical. But, the acquired immune system came with attendant costs that have become evident in the fullness of time as the immune system and parasites engaged in runaway "Red Queen" coevolution. Rather than celebrate the acquired immune system as an optimal solution, we might see it as an appendage that generates its own necessity.

A Changed Perception

The invention of acquired immunity was like escalating a war with an omnipotent opponent, one that just deflects your energy or maybe even turns it back upon you. How does this knowledge affect the way we think about disease and immunity? For many years the study of immunology was carried out with little regard for infectious agents. With apologies to immunopathologists, much of what we've learned about immunology came from studying the immune response in inbred mice to dextran, sheep red blood cells, bovine serum albumin, keyhole limpet hemocyanin, ovalbumin, hen egg lysozyme, or cytochrome c—antigens that don't fight back. Only recently have immunologists, en masse, discovered the way infection with pathogens informs our understanding of the immune response. Even now, the strains of virus or bacteria used are laboratory strains selected to invoke huge responses before being cleared by the immune system of C57BL/6 mice. We do this despite the knowledge that species variation with respect to pathogen resistance is well documented (Sorci et al., 1997; Carius et al., 2001; Smith et al., 1999). C57BL/6 mice clear an infection with *Leishmania* whereas BALB/c mice succumb (Sacks and Noben-Trauth, 2002); yet, the susceptibility pattern of the two mouse strains is exactly the reverse for infection with the parasitic protozoan *Toxoplasma gondii* (Suzuki et al., 1995). Now that we understand quite a lot about the components of the immune system, how would the immune response look different were we to use real mouse pathogens in wild mice? In experimental immunology we attempt to avoid variation as much as possible, but what is the variation in a natural population (or even among strains), and what are the mechanisms employed by variants that confer resistance? A combination of genomics and immunopathology will surely uncover the subtle changes in the immune system that can dramatically affect virulence and the outcome of an infection. Such changes may be identified with the acquired immune system, but a prediction is that they will often be associated with innate immunity.

Given that parasites are so wonderfully diverse, devious, and determined, is a "complete" knowledge of the average immune system the most important goal for human disease control? Very clearly, some diseases lend themselves to control by vaccination, although thus far, our successes have been limited to diseases caused by infectious agents that induce an effective and lasting immune memory. When a parasite has produced a strategy that prevents a memory response, our ability to produce an effective vaccine is thus far, nil. That doesn't mean there is no possibility for an effective vaccine, it just means we face a steep uphill struggle, and perhaps this is where the study of species- and strain-specific resistance may be fruitful.

I conclude that a clear evolutionary approach to the problem of host-pathogen interactions might encourage the field to reconsider some of its most closely held tenets. Perhaps we should not assume that each and every disease can be controlled by vaccination. Considering the biological invention that has been directed toward thwarting T cell responses and antibody reactions, the possibility exists that for some agents, the acquired immune system is not up to the task. Other avenues of treatment might be more efficacious, but in a more fatalistic vein, one might conclude that the most effective means of controlling disease, as it has always been, is public sanitation, vector control, and education. A parasite can't replicate in a host to which it has no access. It is antithetical to biomedical science as practiced in western countries, but technology may not be the answer to most of the world's infectious diseases.

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