

Chapter 1

Evolution of Bacterial-Host Interactions: Virulence and the Immune Overresponse

ELISA MARGOLIS AND BRUCE R. LEVIN

While many people may not believe in evolution, for those of us with the great taste and good fortune to work with bacteria, viruses, and single cell fungi, evolution is not a matter of belief, and much less one of faith. Evolution is something we constantly see whether we want to or not. For those who are evolutionary biologists by training, inclination, or aspiration there is an obligation to be more than just witnesses and historians of evolution. We have to provide explanations for the origin and maintenance of all biological phenomena. There can be no exceptions.

Coming up with these explanations and better yet with testable evolutionary hypotheses is not hard for characters that provide obvious fitness advantages to the organisms that express them. The ascent of resistance following the introduction of antibiotics came as no surprise to evolutionary biologists. In the presence of antibiotics, bacteria that are resistant to their action have an obvious selective advantage relative to their susceptible ancestors. More challenging to account for are situations where it is not clear how the character in question could have evolved by natural selection favoring the individual organisms. While the interactions between parasitic bacteria and their mammalian hosts include many characters that can be explained by natural selection operating at the level of individual bacteria or individual hosts (Burnet and White, 1972), there are many that cannot. Virulence is one of these traits that is hard to account for by simple evolutionary models; why would bacteria harm the hosts they need for their survival?

In this chapter (speculative rant, if you prefer) we focus primarily on aspects of the evolution of the bacterium-host (mostly human) interactions that cannot be readily accounted for by simple, advantage-to-the-individual evolutionary scenarios. We postulate and provide evidence that much of the virulence of bacte-

rial infections can be blamed on the seemingly misguided overresponse of the immune defenses, what is sometimes referred to as “friendly fire” (Levin and Anita, 2001; Whitnack, 1993) or immunopathology (Graham et al., 2005). We consider how this perversity of the immune system fits with current hypotheses for the evolution of virulence, the evolution of the so-called virulence factors, and speculate on the reasons natural selection has failed to or is unable to blunt the immune overresponse to bacterial infections. We conclude with a brief discussion of the implications of this perspective on virulence for the treatment of bacterial infections.

BACTERIAL VIRULENCE AS AN IMMUNE OVERRESPONSE

We define virulence as the magnitude of the morbidity and the increase in the likelihood of mortality resulting from the colonization and proliferation of bacteria in or on a host. To facilitate our consideration of this virulence and its evolution we use the gross simplification, a cartoon, of the bacterium-host interaction presented in Color Plate 1. Bacteria enter a site, the blue box, where they replicate and establish a population and colonize the host, but in which they do not generate perceptible symptoms. Virulence requires their passage into a second site, the red box, where the presence of bacteria (or their products) can, but need not, cause symptoms, e.g., for a *Streptococcus pneumoniae* bacteremia the blue site is the nasopharynx and the red is the bloodstream. In this model the red site needn't be a different physical location. It could be a different state of the bacteria in the site of their colonization, e.g., for a *Staphylococcus aureus* skin infection, the blue site would be the skin and the red a boil.

In Color Plate 1 as well as in mammals, virulence occurs in two ways, both of which require the bacteria to enter the red, potentially symptomatic site or state: (i) direct damage to the host tissue is caused by the replication of the bacteria and/or the production of specific products (toxins), or (ii) indirect damage to the host occurs through an inappropriate or overresponse of the immune system. Both types of damage are represented by the "!" within a triangle. In this scheme the immune defenses can prevent virulence in one or more of seven related ways:

1. Limiting the entry of bacteria into the asymptomatic site
2. Limiting the proliferation of the bacteria in the asymptomatic site
3. Increasing the rate of clearance of bacteria and their products from the asymptomatic site
4. Preventing entry of bacteria or their products into the potentially symptomatic site or state
5. Reducing the rate of proliferation of the bacteria within the potentially symptomatic site or state
6. Increasing the rate of clearance of bacteria and their products from the potentially symptomatic site or state
7. Preventing an immune overresponse to the bacteria or their products in the potentially symptomatic site or state.

The first three of these immune responses maintain the density of bacteria and concentrations of their products in the asymptomatic site at levels where they are unlikely to spill over or otherwise enter the site or state where they can generate symptoms. Whether they do generate symptoms and the magnitude of those symptoms given passage into the red site or state also depends on how well the immune system limits their densities and the concentrations of their products. In Color Plate 1, the number 8 is the infectious transmission of bacteria promoted by the generation of symptoms, and the number 9 is the transmission of bacteria from the asymptomatic site. All of these enumerated steps in which the immune system limits the virulence of the bacteria can be classified as appropriate responses. However, inappropriate responses, for which we use the term *overresponse* when they lead to host damage, may occur due to defects in one of these steps or as a secondary consequence of mounting an immune response.

In the following we focus primarily on the virulence resulting from the overresponse of the immune system. There are, however, examples of virulence that can be attributed to the direct damage of host tissue by the replication of bacteria or the secretion of their products. Included among these are (i) dental caries, resulting from the acid produced by metabo-

lizing *Lactobacillus acidophilus* or *Streptococcus mutans* (Gibbons, 1964), (ii) paralysis due to the neurotoxins secreted by *Clostridium botulinum* or *Clostridium tetani* acting on the nerve and motor endplates (Schiavo et al., 1992), and (iii) diarrhea resulting from enterotoxins that inhibit resorption of sodium chloride or promote its secretion. Examples of virulence being a direct product of the interaction between bacteria and host cells appear to be rare relative to those in which the morbidity and mortality can be attributed to the indirect damage due to an immune overresponse.

As illustrated in Table 1, the morbidity and mortality of bacterial infections can be attributed to the host's immune system operating in one of three inappropriate ways: (i) being more vigorous than needed, (ii) being incorrect for that pathogen, or (iii) responding to the wrong signals. The best-investigated example of the immune system responding too vigorously is bacterial sepsis, where the entry of cytokines and bacteria into the bloodstream brings about widespread blood vessel injury and multiple organ failure (impaired pulmonary, hepatic, or renal function). Here the response to the bacteria is at one level appropriate, as the cytokines released play an important part in attracting neutrophils (immune cells that phagocytose bacteria) to the local infection site, but is also excessive (Kurahashi et al., 1999). The distinction between an inappropriate and appropriate immune response can be seen in the spectrum of illness associated with *Mycobacterium leprae* (Modlin, 2002; Sieling et al., 1999). Hosts that respond to infection predominantly with antibodies and very few CD4 T cells have infectious sites with large macrophages that contain numerous mycobacteria. These macrophages are responsible for the multiple skin lesions and nodules seen in lepromatous leprosy, while a host with T helper 1-type response (high interferon- γ production and low interleukin-4 [IL-4]) has numerous well-formed granulomas with very few mycobacteria that form minor skin lesions. Superantigens provide an example of the immune system responding to an incorrect signal. Superantigens are bacterial products that stimulate a large number of T cells (1–40% of T cells will react) by binding to major histocompatibility complex class II molecules and T cell receptors (beta chain) independently of their specificity for antigens (Rott and Fleischer, 1994). *S. aureus*, *Streptococcus pyogenes*, *Mycoplasma arthritidis*, and *Yersinia pseudotuberculosis* are among the bacteria that produce superantigens. In the case of toxic shock syndrome, the superantigens produced by *S. aureus* induce the indiscriminate and overwhelming activation of T cells leading to the production of cytokines that mediate shock and tissue injury. In all three of these cases the morbidity and mor-

Table 1. Some examples of virulence resulting from an immune overresponse

Disease	Bacteria	Red site ^a	Bacterium-host interaction ^b	Damage induced by immune response
Pneumonia	Multiple species, e.g., <i>S. pneumoniae</i> , <i>N. meningitidis</i> , <i>H. influenzae</i>	Lungs	Cell wall and other bacterial components → induce proinflammatory cytokines, edema, and fibrin deposition (Bergeron et al., 1998)	Recruitment of fluid and cells into the air spaces of the lungs
Toxic shock syndrome/scarlet fever	<i>Staphylococcus</i> , <i>Streptococcus</i>	Circulatory system, systemic	Superantigens → indiscriminate activation of T cells (McCormick et al., 2001)	Extreme inflammation leading to septic shock
Duodenal ulcers	<i>Helicobacter pylori</i>	Gastric and duodenal mucosa	Bacterial persistence → chronic inflammation (Czinn and Nedrud, 1997)	Mucosal atrophy
Septicemic plague	<i>Yersinia pestis</i>	Circulatory system, systemic	Endotoxin and/or unknown factors → systemic inflammatory response	Acute shock
Cutaneous anthrax	<i>Bacillus anthracis</i>	Skin	Anthrax lethal factor (toxin) → release of TNF- α and IL1 β (Firoved et al., 2005)	Tissue necrosis
Petechiae, Waterhouse-Friderichsen syndrome	<i>Neisseria meningitidis</i>	Skin and adrenal glands	Endotoxin → release of TNF- α , IL-1, and IL-6 (Klein et al., 1996)	Blood vessel destruction
Rheumatic fever	<i>Streptococcus pyrogenes</i>	Heart, joints, skin and brain	M protein → activation of autoreactive (anticardiac myosin) antibodies (Cunningham, 2003)	Antibody-induced damage of heart muscle; immune complex deposited in large joints and skin
Reactive arthritis	<i>Chlamydia trachomatis</i>	Joints, eyes, urethra	Bacterial persistence → activation of autoreactive immune cells (Sieper, 2001)	Inflammation of large joints, eyes, and urethra
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Lungs	Bacterial presence → release of high levels of TNF- α (Bekker et al., 2000; Kaushal et al., 2002)	Recruitment of fluid and cells into the air spaces of the lungs; necrosis
Meningitis	Multiple species, e.g., <i>S. pneumoniae</i> , <i>N. meningitidis</i> , <i>H. influenzae</i>	Meninges	Cell wall and other bacterial components → induce proinflammatory cytokines and chemokines (Braun et al., 1999)	Increased blood-brain barrier permeability; neutrophil recruitment; increased intracranial pressure; brain damage

^aSee Fig. 1 for an explanation of red site.

^bTNF- α , tumor necrosis factor alpha.

tality of the host can be attributed to an apparently misguided response of the immune system, which we refer to as an overresponse.

THE EVOLUTION OF BACTERIAL VIRULENCE AS AN IMMUNE OVERRESPONSE

How does the observation that much of the morbidity and mortality can be attributed to a host overresponse to bacteria help in understanding the evolution of virulence? In a perspective written a decade ago, one of us (Levin, 1996) listed four hypotheses

that account for the evolution of virulence: (i) the conventional wisdom, (ii) epidemiological selection, (iii) coincidental evolution, and (iv) short-sighted, within-host evolution. Since that time, although there have been a number of theoretical, experimental, and speculative articles on the evolution of virulence (for a small and admittedly biased sample see Andre and Godelle, 2006; Bonhoeffer and Nowak, 1994; Brown et al., 2006; Bull, 1994; Elbert and Bull, 2003; Ebert and Herre, 1996; Frank, 1996; Grech et al., 2006; Lipsitch et al., 1995, 1996; Lipsitch and Moxon, 1997; Mackinnon and Read, 2004; Regoes et al., 2000), we do not know of studies that have rejected any of these

hypotheses and only one adding what may be a new hypothesis: quasispecies evolution (Pfeiffer and Kirkegaard, 2005). This fifth hypothesis may only apply to viruses with high mutation rates and arguably could be subsumed under the broader rubric of within-host evolution. In this section we consider how the observation that morbidity and mortality of bacterial infections can be attributed to the hosts' immune over-response fits each of these hypotheses for the evolution of the virulence of bacteria.

The Conventional Wisdom

This phrase, which the late John Kenneth Galbraith coined to describe ideas and explanations that are widely accepted as true by the public, was applied by Bob May and Roy Anderson (May and Anderson, 1983b) to describe the then-prevailing view of the evolution of the virulence. According to that wisdom, virulence is an artifact of the relative novelty of parasite's association with its host. As the relationship between the parasite and host matures, natural selection in either the parasite or host population or both will lead to the extinction of one or the other species or the evolution of symbiosis or mutualism.

While the original theory behind this hypothesis for the evolution of virulence of infections amounts to little more than the adage "don't bite the hand that feeds you," the evidence in support of it was and remains compelling. Many of the bacteria responsible for morbidity and mortality of humans were acquired from other species in the not-so-distant past (after the advent of agriculture), and some are continuously acquired in this way. Included among these zoonotic (and protozoonotic) infections are plague, tuberculosis, Legionnaires' disease, botulism, anthrax, brucellosis, tularemia, Rocky Mountain spotted fever, cholera, and other diarrheal diseases. The bacteria responsible for some of these infections, such as *Mycobacterium tuberculosis*, are transmitted between humans and can be maintained without the animal source. Others such as *Legionella pneumophila* are not. Also consistent with the conventional wisdom is the correlated observations that only a very small minority of the vast numbers of species of bacteria that colonize mammals cause disease.

It may seem that the proposition that the virulence of bacterial infections can be attributed to host immune over-response fits quite well with this conventional wisdom. To wit, the immune system has not yet had the time to evolve to moderate the response to these novel bacteria and their products and/or these bacteria have not yet evolved into being nice. Eventually, or as it was once referred to, on "equilibrium day," (Levin et al., 2000), mutualism will prevail and the immune over-response will be tempered.

Epidemiological Selection

The conventional wisdom is an observation rather than a mechanism, an observation that focuses on the interactions between bacteria and the individual hosts they colonize. To fully understand the evolution of commensal and pathogenic bacteria, however, it is necessary to consider their lifestyle outside the host and, in particular, their transmission between hosts. One approach to this more comprehensive picture of the evolution of parasitic microbes has been to draw inferences about the nature and direction of selection from epidemiological models (Levin et al., 1982; Levin and Pimentel, 1981; May and Anderson, 1983a). In accord with this perspective, the fitness of a particular strain of bacteria is given by its basic reproductive number, R_0 , the number of secondary infections caused by a single infected individual in a wholly susceptible population of hosts; the higher the value of R_0 , the greater the fitness of the bacteria. In these traditional epidemiological models, virulence is only expressed as mortality. Morbidity and other more subtle effects of infections are not directly considered in these epidemiological models.

As long as the transfer to new hosts requires viable hosts, selection will favor bacteria that are not only infectiously transmitted at ever-higher rates but also persist longer in colonized hosts (i.e., are less likely to kill the host). In other words, selection will favor ever-more-benign, symbiotic, or better yet, mutualistic bacteria. Evolution in the host population will also be for reduced virulence; hosts that are less subject to infection-associated morbidity and mortality will be favored. As long as transmission occurs from the blue asymptomatic site (8 in Color Plate 1) rather than the red site (9 in Color Plate 1), these epidemiological models can be seen as the theoretical basis of the conventional wisdom (also see Lenski and May, 1994). If, however, transmission and the morbidity and mortality of the host are coupled so the more virulent bacteria are transmitted at higher rates than the more benign, there is a trade-off between the loss of the host and gain to the bacteria; virulence would be favored in the bacterial population (Ebert and Bull, 2003).

On first consideration it may seem that this transmission and virulence trade-off is inconsistent with the proposition that the morbidity and mortality of the infection is a product of the host's immune over-response. We suggest this is not necessarily the case. The host over-response could be a by-product of selection operating on individual bacteria to promote their transmission. While we don't know of overwhelming, quantitative, empirical evidence of this being the case for any pathogenic bacteria (viruses are

another matter; see Fenner and Ratcliffe, 1965), this interpretation is supported by reasonable plausibility arguments. Here we consider two of the more compelling of these examples of pathogenic bacteria of humans.

The first is the diarrheal diseases in which humans play a significant role in the transmission process. Because of the massive output of bacteria, diarrhea is likely to increase the density of bacteria in water and food products and thereby the transmission rate of these bacteria. Thus, as long as transmission is promoted by diarrhea, selection in the bacterial population will favor mechanisms that cause diarrhea. In some cases the induction of diarrhea is attributed to what can be seen as immune overresponse. The dysentery bacteria *Shigella flexneri* induces the release of the cytokine interleukin 1 (IL-1) in infected macrophages, which leads to extensive injury of the colon mucosa, which in turn results in fluid and protein loss into the intestinal lumen and the ensuing diarrhea (Hilbi et al., 1997). This hypothesis for the evolution of diarrhea to increase transmission requires that the transmission advantage more than makes up for the loss in transmission due to host mortality. To our knowledge, there are no quantitative empirical studies demonstrating that this trade-off obtains for any diarrheal disease.

The second example is plague. Albeit not yet as well documented as the oft-told mother of all trade-off stories, myxoma and the Australian rabbits (Fenner, 1965), the emerging tale of the evolution of the virulence of the plague bacillus, *Yersinia pestis*, has parallels to that story. There is compelling evidence that this flea-transmitted pathogen evolved from a not very virulent enteric, oral-fecal transmitted *Yersinia* relatively recently by the acquisition of a couple of plasmids and a few chromosomal genes (Achtman et al., 1999; Carniel, 2003). Since fleas acquire these bacteria from the blood of rodents, the density of bacteria in circulating blood would be directly associated with the likelihood of their transmission to other rodents (or humans). Also directly associated with this density of bacteria in the blood is sepsis, the virulent manifestation of *Y. pestis* infections. Elisabeth Carniel (personal communication) has suggested that the capacity to generate lethal sepsis is not just a by-product of the proliferation of bacteria in the blood, but may be selected for in the bacterial population. Although the cost-benefit calculation has not been made, it may be that the rate of transmission of the bacteria is augmented by their killing infected rodents, as fleas move to new hosts when their original host dies. For both diarrheal diseases and plague, the virulence resulting from the host overresponse is associated with transmission. Clearly more empirical

work would be necessary to confirm the existence of a trade-off between bacterial transmission and an immune overresponse and the postulated exploitation of this overresponse for the epidemiological advantage of the parasite.

Coincidental Evolution

In accord with this hypothesis there is no advantage to the bacteria to make the host sick and certainly no advantage for the host to be ill; virulence is a consequence of the bacteria being in the wrong host or in a wrong site in the right host (Levin and Svanborg Eden, 1990) (the arrow above 7 in Color Plate 1). The bacterial products responsible for the morbidity and/or mortality of the host, virulence determinants, evolved in response to selection for some function other than virulence.

Reasonable candidates for coincidental virulence due to an immune overresponse are diseases associated with *Helicobacter pylori*. These bacteria colonize and maintain populations in the stomachs of the majority of humans for most of their lives without generating symptoms and appear to have done so since prehistoric times (Falush et al., 2003). However, it wasn't until Marshall and Warren (1984) presented evidence that a curved bacteria we now know as *H. pylori* was an etiologic agent for gastric and peptic ulcers that this seemingly commensal bacteria was elevated to the status of pathogen. This distinction was further enhanced by evidence that *H. pylori* was also associated with gastric cancers (Moss and Blaser, 2005; Tatematsu et al., 2005). *H. pylori* colonization can result in a chronic inflammatory state that is generated when the host responses (such as the release of IL-8 and other chemokines, the attraction of neutrophils, macrophages, and the local stimulation of T cells) fails to clear the bacteria and lymphoid aggregates form in the lamina propria of the stomach and duodenum. This continued stimulation of the immune and inflammatory cells (termed chronic atrophic gastritis) results in the destruction of the gastric epithelium, formation of peptic ulcers, and increased risk for gastric cancers. Presumably, but not yet formally demonstrated, the induction of the inflammatory response and the subsequent diseases provides no advantage to *H. pylori* in a colonized host or its transmission to new hosts. In this sense, the virulence of *H. pylori* in colonized humans is coincidental.

While they are commonly described as pathogens, especially in grant proposals and by people suffering from the symptoms they can generate, a number of bacteria responsible for morbidity and mortality in humans also have good credentials as commensals.

Like *H. pylori* they are carried asymptotically by many and cause disease in few. Included among the more prominent of these commensal pathogens for humans are *S. aureus*, *Haemophilus influenzae*, *S. pneumoniae*, and *Neisseria meningitidis*. From an evolutionary perspective, invasive disease seems to be the wrong thing for these bacteria to do—dead ends. The sites of their virulence, blood and meninges, are certainly not good for their transmission to new hosts by their normal route, through respiratory droplets. The rare virulence of these commensal bacteria can be accounted for by an immune overresponse in these sites (Bergeron et al., 1998; Braun et al., 1999). The occasional movement of bacteria into a site where they can cause disease (the red in Color Plate 1) may be due to chance or coincidental evolution or as we argue below may be a consequence of within-host evolution of the bacterial population.

Within-Host Evolution

In accord with this hypothesis, the virulence of bacteria is the product of selection favoring more pathogenic members of a population colonizing an individual host (Levin and Bull, 1994). The advantage gained by the bacteria by generating symptoms in a colonized host is restricted to that host and may be to its disadvantage in its transmission to a new host; this evolution is short-sighted. A mutant commensal bacterium with the capacity to establish and maintain populations in normally sterile sites, cells, or tissues could be favored within a colonized host because in those sites there is less competition for nutrients and/or those mutant bacteria are somewhat protected from the host immune defenses.

Although we can make a good case and even cite evidence for the virulence of some viruses, such as poliovirus and Cocksackievirus, being the product of within-host evolution (Gay et al., 2006; Levin and Bull, 1994), for bacteria the best we can do at this stage is present arguments founded on plausibility and consistency with observations (see, for example, Meyers et al., 2003). Central to these arguments are the results of studies with mice and rats demonstrating that the bacteria responsible for invasiveness (blood infection) are commonly derived from one of very few cells (Meynell, 1957; Moxon and Murphy, 1978; Pluschke et al., 1983; Rubin, 1987). One possible explanation for these observations is that the bacteria responsible for the blood infections are the products of single, mutant cells with an enhanced capacity to invade and proliferate in blood.

While supporting the within-host evolution hypothesis for virulence, these observations are also consistent with the coincidental evolution hypothesis:

that, by chance alone, only one or a few cells establish blood infections can be attributed to very small holes in the host's defenses through which only one or very few bacteria traverse the arrow above 7 in Color Plate 1. Although the coincidental and within-host hypotheses could be distinguished by demonstrating that the bacteria establishing a blood infection have an inherited propensity for the invasion of blood, to our knowledge there are no published studies that have done this test. However, whether the invasiveness of the blood or other normally sterile sites is coincidental or due to within-host evolution, the virulence of bacteria in these sites can be attributed to a host's immune overresponse.

The Evolution of Virulence Determinants

Not all bacteria or even all members of the same species of bacteria capable of colonizing mammals are responsible for disease. One explanation for why some bacteria cause disease and others do not is what have become known as virulence factors or virulence determinants, the expression of which are, by definition, essential for that bacteria to cause disease in (or on) colonized hosts (Finlay and Falkow, 1989). Included among these are characters that facilitate adhesion to host cells, evade the host constitutive and inducible immune defenses, and produce toxins. Appropriately, much of contemporary bacteriology is devoted to understanding the molecular biology, genetics, evolutionary origin, and mode of action of virulence determinants as a way to understand bacterial diseases and ideally prevent or treat them. While virulence determinants (factors) are almost certainly the products of adaptive evolution in bacterial populations, not so clear are the selection pressures responsible for their evolution and maintenance. Are they favored because of virulence, i.e., the morbidity and mortality of the host promotes the colonization, persistence, and infectious transmission of bacteria that express these determinants? Are virulence factors by-products of selection for other functions, e.g., their expression provides protection against grazing protozoa (Wildschutte et al., 2004) and/or facilitates competition with other microbes? Or is the virulence attributed to these factors an inadvertent by-product of their normal function in a host, a primitive character that will be lost on or before equilibrium day. While these hypotheses may be mutually exclusive for any specific bacterium-host and virulence factor, they are clearly not so collectively. Whether they evolve in response to selection for virulence or not, some of these virulence factors are responsible for triggering the immune overresponse.

Why Does the Immune System Overrespond?

In the preceding, we have portrayed the host immune system as misguided, overresponding in ways that cause rather than prevent the morbidity and mortality of a bacterial infection. From the perspective of evolutionary biology, however, "misguided" is hardly an explanation. Colonization by bacteria is not a rare event but rather something mammals confront all the time, and overresponding in a way that results in their morbidity and mortality would almost certainly be selected against. In their review of "immunopathology," Graham and colleagues postulated a number of reasons for this transgression of the immune response (Graham et al., 2005). Here we offer our perspective on this issue.

As we see it, there are two general classes of explanations for the maintenance of an overresponse of the immune system. (i) While infectious disease may be a major source of morbidity and mortality (Haldane, 1949), disease-mediated selection can be relatively weak, and extensive amounts of time would be required to evolve mechanisms to modulate the immune response to specific bacterial infections. (ii) Functional constraints on the immune system limit the ability of natural selection to totally prevent and maybe even partially mitigate an immune overresponse to bacterial infections.

(a) Even if selection universally favors tempering the immune overresponse to infections, and the favored genotypes could be generated (which we question below [b]), the time required for temperance to evolve could be considerable, especially if the overresponse is specific for particular bacteria and/or their products. This is due to two factors. (a) At its maximum the intensity of selection for modulating the immune overresponse to an infection would equal the fraction of the population with that infection. It would be substantially lower if the symptoms of the infection were not expressed in all colonized hosts, were rarely lethal or sterilizing, or were primarily manifest after reproductive years or if the magnitude of the reduction of the overresponse of the favored genotype was less than absolute. For most of the diseases listed in Table 1 virulence is a rare occurrence in colonized hosts (less than 1%), and therefore the intensity of selection against an immune overresponse would be relatively weak. (b) It can take a considerable amount of time for a rare beneficial mutant to ascend to substantial frequencies. For example, if the selection for a reduced overresponse is operating on genotypes at a single locus (the best case), the initial frequency of a favored allele is 10^{-3} , the favored genotype has a 1% selective advantage, and there is no dominance, it would take 1,381 generations (more than 20,000 years for humans) for that gene to reach

a gene frequency of 50%. If the favored genotype is recessive, the corresponding number of generations would be 100,491 (Crow and Kimura, 1971).

What about the role of the bacteria in the evolution of a more temperate immune system? As a consequence of their vastly shorter generation times, haploid genomes, and propensity to receive genes and pathogenicity islands by horizontal transfer, it seems reasonable to assume that bacteria would have an edge in an evolutionary arms race with their mammalian hosts. We suggest, however, that this edge contributes little if anything to the slowing pace at which mammalian evolution could modulate the immune overresponse. Although there maybe situations where virulence is positively correlated with the infectious transmission of bacteria, in most of these cases the morbidity and mortality associated with their transmission is not to the bacteria's advantage and may be to their disadvantage. Even greater transmission of these bacteria would be possible if the hosts were not debilitated or killed as a result of diarrhea or if the bacteremias required for vector-borne transmission did not result in sepsis. In this interpretation evolution in the bacteria population would not oppose the evolution of a more temperate host immune system. Of all the examples considered in this chapter, the only one in which evolution in the bacterial population might favor an immune overresponse is Carniel's suggestion that by killing their host, *Y. pestis* acquires a transmission advantage.

(ii) While the above realities of the ecology and genetics of natural selection may be part of the answer to the question of why evolution has not eliminated the immune system's overresponse to bacterial (and other) infections, we suggest it is not the most important reason. We conjecture that the primary reason mammalian evolution has not tempered and perhaps cannot temper the immune overresponse to bacterial and other infections is functional constraints that limit the extent to which the immune system can be modified. The immune system has roles other than clearing bacterial infections. It has been postulated that these other roles dominated the evolution of the mammalian immune system (Burnet, 1970). These different roles as well as the extraordinary diversity of organisms colonizing mammals, bacteria, viruses, fungi, and worms of various ilks and the variety of sites of colonization impose different and potentially conflicting demands on the immune defenses, phenomena referred to as antagonistic pleiotropy. An appealing hypothesis for the immunopathology known as allergies is an overresponse of those elements of the immune system that in less-pristine times would otherwise be occupied with the control of helminth infections (Wilson and Maizels, 2004).

There is a fine line between responding (1–6 in Color Plate 1) and overresponding (7 in that figure), which may be difficult for the systems regulating the immune response to perceive, much less avoid. As suggested by Frank (Andre et al., 2004), the intensity of an immune response may be determined by a trade-off between increasing the strength and rapidity of an immune defense and the virulence from an immune system overresponse.

Is there evidence in support of these two hypotheses for why evolution has not eliminated the virulence resulting from the immune overresponse? Not much—at least not yet. We suggest, however, that some of the considerable amount of inherited variability in the susceptibility to infectious disease in human populations (Bellamy et al., 2000; Bellamy and Hill, 1998; Segal and Hill, 2003; Sorensen et al., 1988) can be interpreted as support for these hypotheses. To be sure, there is good and even overwhelming evidence that some of this variation is maintained by disease-mediated balancing or frequency-dependent selection, but this is not the case for all or even the majority of it. We suggest that much of the standing genetic variation in disease susceptibility in human populations is a reflection of the myopia and limitations of natural selection: (i) the relative weakness of selection for modulating the immune overresponse and (ii) even more, the impotency of natural selection due to the constraints on the immune system—antagonistic pleiotropy. Genetic variation that is not or is poorly perceived by natural selection will build up and persist (Crow and Kimura, 1971).

Implications

While the morbidity and mortality of most bacterial infections can be attributed to an immune overresponse, virtually all of our efforts to treat these infections are directed at controlling the proliferation and clearing the bacteria, primarily with antibiotics. This approach has been and continues to be effective, but not completely so. Antibiotic treatment commonly fails, and patients die or remain ill for extended periods. Resistance of the pathogen to the antibiotics employed for treatment is only one of the reasons for this failure and for some infections is not the major one, at least not yet (Levin and Rozen, 2006; Yu et al., 2003).

The obvious alternative approach to treating infections is to reduce the morbidity and prevent the mortality by modulating the immune system's overresponse. There have been attempts to do just that for the treatment of bacteria-mediated sepsis. Clinical trials have evaluated the use of glucocorticoids (Bone et al., 1987), drugs designed to neutralize endotoxins

(Ziegler et al., 1991), tumor necrosis factor α (Fisher et al., 1996), and IL-1 β (Fisher et al., 1994), but none of these treatments was effective. The most successful trials in humans to date have been with a component of the natural anticoagulant system, activated protein C, which has substantial anti-inflammatory properties along with being a potent anticoagulant (reduces the formation of clots that are responsible for organ failure in late stages of sepsis) (Fourrier, 2004). In addition, new agents redirect the immune response and hold promise as effective future therapies for sepsis, such as IL-12 (O'Suilleabhain et al., 1996) and antibodies against complement (C5a) (Czermak et al., 1999). However, understanding the specifics of the immune overreaction and the intricacies of the feedback mechanisms that control an immune response is necessary for therapies to be directed at enhancing or inhibiting the patient's immune response.

At this time, taken at large, the success of these immune modulating methods in preventing the morbidity and mortality of bacterial infections can at the very best be described as modest. However, in maintaining the speculative nature of this rant, and desiring an optimistic conclusion, we suggest that as we learn more about the regulation of the immune response and develop procedures to monitor as well as administer regulatory immune molecules in real time, these methods will become increasingly effective for the treatment of bacterial infection.

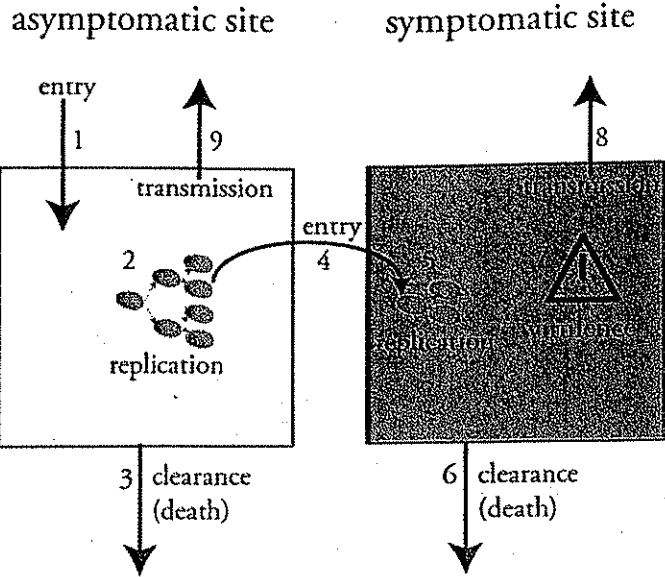
Acknowledgments. We thank Elisabeth Carniel for sharing her ideas about the evolution of the virulence of *Y. pestis*. We are grateful to Jim Bull and Harris Fienberg for insightful comments and suggestions. B.R.L. acknowledges his continuous gratitude to Fernando Baquero, for inspiration, ideas, never-ending whimsy, support, and friendship. This endeavor was supported by a grant from the NIH, AI40662 (B.R.L.), and an NIH Training Grant (E.M.).

REFERENCES

- Achtman, M., K. Zurth, G. Morelli, G. Torrea, A. Guiyoule, and E. Carniel. 1999. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proc. Natl. Acad. Sci. USA* 96:14043–14048.
- Andre, J.-B., S. Gupta, S. Frank, and M. Tibayrenc. 2004. Evolution and immunology of infectious diseases: what new? An E-debate. *Infect. Genet. Evol.* 4:69–75.
- Andre, J. B., and B. Godelle. 2006. Within-host evolution and virulence in microparasites. *J. Theor. Biol.* 241:402–409.
- Bekker, L. G., A. L. Moreira, A. Bergtold, S. Freeman, B. Ryffel, and G. Kaplan. 2000. Immunopathologic effects of tumor necrosis factor alpha in murine mycobacterial infection are dose dependent. *Infect. Immun.* 68:6954–6961.
- Bellamy, R., N. Beyers, K. P. McAdam, C. Ruwende, R. Gie, P. Samaai, D. Bester, M. Meyer, T. Corrah, M. Collin, D. R. Camidge, D. Wilkinson, E. Hoal-Van Helden, H. C. Whittle, W. Amos, P. van Helden, and A. V. Hill. 2000. Genetic susceptibility to tuberculosis in Africans: a genome-wide scan. *Proc. Natl. Acad. Sci. USA* 97:8005–8009.

- Bellamy, R., and A. V. Hill. 1998. Genetic susceptibility to mycobacteria and other infectious pathogens in humans. *Curr. Opin. Immunol.* 10:483-487.
- Bergeron, Y., N. Ouellet, A. M. Deslauriers, M. Simard, M. Olivier, and M. G. Bergeron. 1998. Cytokine kinetics and other host factors in response to pneumococcal pulmonary infection in mice. *Infect. Immun.* 66:912-922.
- Bone, R. C., C. J. Fisher, Jr., T. P. Clemmer, G. J. Slotman, and C. A. Metz. 1987. Early methylprednisolone treatment for septic syndrome and the adult respiratory distress syndrome. *Chest* 92:1032-1036.
- Bonhoeffer, S. A., and M. A. Nowak. 1994. Mutation and the evolution of virulence. *Proc. R. Soc. London B* 258:133-140.
- Braun, J. S., R. Novak, K. H. Herzog, S. M. Bodner, J. L. Cleveland, and E. I. Tuomanen. 1999. Neuroprotection by a caspase inhibitor in acute bacterial meningitis. *Nat. Med.* 5:298-302.
- Brown, N. F., M. E. Wickham, B. K. Coombes, and B. B. Finlay. 2006. Crossing the line: selection and evolution of virulence traits. *PLoS Pathog.* 2:e42.
- Bull, J. J. 1994. Virulence. *Evolution* 48:1423-1437.
- Burnet, F. 1970. *Immunological Surveillance*. Pergamon Press, Oxford, United Kingdom.
- Burnet, F. M., and D. O. White. 1972. *Natural History of Infectious Diseases*. Cambridge University Press, Cambridge, United Kingdom.
- Carniel, E. 2003. Evolution of pathogenic *Yersinia*: some lights in the dark. *Adv. Exp. Med.* 529:3-12.
- Crow, J. F., and M. Kimura. 1971. *An Introduction to Population Genetics Theory*, 1st ed. Harper & Row, New York, NY.
- Cunningham, M. W. 2003. Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. *Front Biosci.* 8:S533-S543.
- Czermak, B. J., V. Sarma, C. L. Pierson, R. L. Warner, M. Huber-Lang, N. M. Bless, H. Schmal, H. P. Friedl, and P. A. Ward. 1999. Protective effects of C5a blockade in sepsis. *Nat. Med.* 5:788-792.
- Czinn, S. J., and J. G. Nedrud. 1997. Immunopathology of *Helicobacter pylori* infection and disease. *Springer Semin. Immunopathol.* 18:495-513.
- Ebert, D., and J. J. Bull. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends Microbiol.* 11:15-20.
- Ebert, D., and E. A. Herre. 1996. The evolution of parasitic diseases. *Parasitol. Today* 12:96-101.
- Falush, D., T. Wirth, B. Linz, J. K. Pritchard, M. Stephens, M. Kidd, M. J. Blaser, D. Y. Graham, S. Vacher, G. I. Perez-Perez, Y. Yamaoka, F. Megraud, K. Otto, U. Reichard, E. Katzowitz, X. Wang, M. Achtman, and S. Suerbaum. 2003. Traces of human migrations in *Helicobacter pylori* populations. *Science* 299:1582-1585.
- Fenner, F., and F. N. Ratcliffe. 1965. *Myxomatosis*. Cambridge University Press, Cambridge, United Kingdom.
- Finlay, B. B., and S. Falkow. 1989. Common themes in microbial pathogenicity. *Microbiol. Rev.* 53:210-230.
- Firoved, A. M., G. F. Miller, M. Moayeri, R. Kakkar, Y. Shen, J. F. Wiggins, E. M. McNally, W. J. Tang, and S. H. Leppla. 2005. *Bacillus anthracis* edema toxin causes extensive tissue lesions and rapid lethality in mice. *Am. J. Pathol.* 167:1309-1320.
- Fisher, C. J., Jr., J. M. Agosti, S. M. Opal, S. F. Lowry, R. A. Balk, J. C. Sadoff, E. Abraham, R. M. Schein, and E. Benjamin. 1996. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N. Engl. J. Med.* 334:1697-1702.
- Fisher, C. J., Jr., G. J. Slotman, S. M. Opal, J. P. Pribble, R. C. Bone, G. Emmanuel, D. Ng, D. C. Bloedow, and M. A. Catalano. 1994. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. *Crit. Care Med.* 22:12-21.
- Fourrier, F. 2004. Recombinant human activated protein C in the treatment of severe sepsis: an evidence-based review. *Crit. Care Med.* 32:S534-S541.
- Frank, S. A. 1996. Models of parasite virulence. *Q. Rev. Biol.* 71:37-78.
- Gay, R. T., S. Belisle, M. A. Beck, and S. N. Meydani. 2006. An aged host promotes the evolution of avirulent coxsackievirus into a virulent strain. *Proc. Natl. Acad. Sci. USA* 103:13825-13830.
- Gibbons, R. J. 1964. Bacteriology of dental caries. *J. Dent. Res.* 43(Suppl):1021-1028.
- Graham, A. L., J. E. Allan, and A. F. Read. 2005. Evolutionary causes and consequences of immunopathology. *Annu. Rev. Ecol. Evol. Syst.* 36:373-397.
- Grech, K., K. Watt, and A. F. Read. 2006. Host-parasite interactions for virulence and resistance in a malaria model system. *J. Evol. Biol.* 19:1620-1630.
- Haldane, J. B. S. 1949. Disease and evolution. *Ric. Sci.* 19:68-76.
- Hilbi, H., A. Zychlinsky, and P. J. Sansonetti. 1997. Macrophage apoptosis in microbial infections. *Parasitology* 115(Suppl):S79-S87.
- Kaushal, D., B. G. Schroeder, S. Tyagi, T. Yoshimatsu, C. Scott, C. Ko, L. Carpenter, J. Mehrotra, Y. C. Manabe, R. D. Fleischmann, and W. R. Bishai. 2002. Reduced immunopathology and mortality despite tissue persistence in a *Mycobacterium tuberculosis* mutant lacking alternative sigma factor, SigH. *Proc. Natl. Acad. Sci. USA* 99:8330-8335.
- Klein, N. J., C. A. Ison, M. Peakman, M. Levin, S. Hammer-schmidt, M. Frosch, and R. S. Heyderman. 1996. The influence of capsulation and lipooligosaccharide structure on neutrophil adhesion molecule expression and endothelial injury by *Neisseria meningitidis*. *J. Infect. Dis.* 173:172-179.
- Kurahashi, K., O. Kajikawa, T. Sawa, M. Ohara, M. A. Gropper, D. W. Frank, T. R. Martin, and J. P. Wiener-Kronish. 1999. Pathogenesis of septic shock in *Pseudomonas aeruginosa* pneumonia. *J. Clin. Invest.* 104:743-750.
- Lenski, R. E., and R. M. May. 1994. The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *J. Theor. Biol.* 169:253-265.
- Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. *Emerg. Infect. Dis.* 2:93-102.
- Levin, B. R., A. C. Allison, H. J. Bremermann, L. L. Cavalliforza, B. C. Clarke, R. Frenzels-Beymem, W. D. Hamilton, S. A. Levin, R. M. May, and H. R. Thieme. 1982. Evolution of parasite systems (group report), p. 212-243. In R. M. Anderson and R. M. May (ed.), *Population Biology of Infectious Diseases*. Springer, Berlin, Germany.
- Levin, B. R., and R. Antia. 2001. Why we don't get sick: the within-host population dynamics of bacterial infections. *Science* 292:1112-1125.
- Levin, B. R., and J. J. Bull. 1994. Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* 2:76-81.
- Levin, B. R., V. Perrot, and N. Walker. 2000. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* 154:985-997.
- Levin, B. R., and D. E. Rozen. 2006. Non-inherited antibiotic resistance. *Nat. Rev. Microbiol.* 4:556-562.
- Levin, B. R., and C. Svanborg Eden. 1990. Selection and evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* 100:S103-S115.
- Levin, S. A., and D. Pimentel. 1981. Selection of intermediate rates of increase in parasite host systems. *Am. Nat.* 117:308-315.

- Lipsitch, M., E. A. Herre, and M. A. Nowak. 1995. Host population structure and the evolution of parasite virulence: a "law of diminishing returns." *Evolution* 49:743-748.
- Lipsitch, M., and E. R. Moxon. 1997. Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* 5:31-37.
- Lipsitch, M., S. Siller, and M. A. Nowak. 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* 50:1729-1741.
- Mackinnon, M. J., and A. F. Read. 2004. Virulence in malaria: an evolutionary viewpoint. *Philos. Trans. R. Soc. London B* 359: 965-986.
- Marshall, B. J., and J. R. Warren. 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1:1311-1315.
- May, R. M., and R. M. Anderson. 1983a. Epidemiology and genetics in the coevolution of parasite and hosts. *Proc. R. Soc. London B* 219:281-313.
- May, R. M., and R. M. Anderson. 1983b. Parasite-host coevolution, p. 186-206. In D. J. Futuyama and M. Slatkin (ed.), *Coevolution* Sinauer, Sunderland, MA.
- McCormick, J. K., J. M. Yarwood, and P. M. Schlievert. 2001. Toxic shock syndrome and bacterial superantigens: an update. *Annu. Rev. Microbiol.* 55:77-104.
- Meyers, L. A., B. R. Levin, A. R. Richardson, and I. Stojiljkovic. 2003. Epidemiology, hypermutation, within-host evolution and the virulence of *Neisseria meningitidis*. *Proc. Biol. Sci.* 270:1667-1677.
- Meynell, G. G. 1957. The applicability of the hypothesis of independent action to fatal infections in mice given *Salmonella typhimurium* by mouth. *J. Gen. Microbiol.* 16:396-404.
- Modlin, R. L. 2002. Learning from leprosy: insights into contemporary immunology from an ancient disease. *Skin Pharmacol. Appl. Skin Physiol.* 15:1-6.
- Moss, S. E., and M. J. Blaser. 2005. Mechanisms of disease: inflammation and the origins of cancer. *Nat. Clin. Pract. Oncol.* 2:90-97 (quiz 1 p. following 113).
- Moxon, E. R., and P. A. Murphy. 1978. *Haemophilus influenzae* bacteremia and meningitis resulting from the survival of a single organism. *Proc. Nat. Acad. Sci. USA* 75:1534-1536.
- O'Suilleabhain, C., S. T. O'Sullivan, J. L. Kelly, J. Lederer, J. A. Mannick, and M. L. Rodrick. 1996. Interleukin-12 treatment restores normal resistance to bacterial challenge after burn injury. *Surgery* 120:290-296.
- Pfeiffer, J. K., and K. Kirkegaard. 2005. Increased fidelity reduces poliovirus fitness and virulence under selective pressure in mice. *PLoS Pathog.* 1:e11.
- Pluschke, G., A. Mercer, B. Kusecek, A. Pohl, and M. Achtman. 1983. Induction of bacteremia in newborn rats by *Escherichia coli* K1 is correlated with only certain O (lipopolysaccharide) antigen types. *Infect. Immun.* 39:599-608.
- Regoes, R. R., M. A. Nowak, and S. Bonhoeffer. 2000. Evolution of virulence in a heterogeneous host population. *Evolution* 54:64-71.
- Rott, O., and B. Fleischer. 1994. A superantigen as virulence factor in an acute bacterial infection. *J. Infect. Dis.* 169:1142-1146.
- Rubin, L. G. 1987. Bacterial colonization and infection resulting from multiplication of a single organism. *Rev. Infect. Dis.* 9: 488-493.
- Schiavo, G., F. Benfenati, B. Poulain, O. Rossetto, P. Polverino de Laureto, B. R. DasGupta, and C. Montecucco. 1992. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* 359:832-835.
- Segal, S., and A. V. Hill. 2003. Genetic susceptibility to infectious disease. *Trends Microbiol.* 11:445-448.
- Sieling, P. A., D. Jullien, M. Dahlem, T. F. Tedder, T. H. Rea, R. L. Modlin, and S. A. Porcelli. 1999. CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. *J. Immunol.* 162:1851-1858.
- Sieper, J. 2001. Pathogenesis of reactive arthritis. *Curr. Rheumatol. Rep.* 3:412-418.
- Sorensen, T. I., G. Nielson, P. Anderson, and T. Teasdale. 1988. Genetic and environmental influences on premature death in adult adoptees. *N. Engl. J. Med.* 318:727-732.
- Tatematsu, M., T. Tsukamoto, and T. Mizoshita. 2005. Role of *Helicobacter pylori* in gastric carcinogenesis: the origin of gastric cancers and heterotopic proliferative glands in Mongolian gerbils. *Helicobacter* 10:97-106.
- Whitnack, E. 1993. Sepsis, p. 770-778. In M. Schaechter, G. Medhoff, and B. I. Eisenstein (ed.), *Mechanisms of Microbial Disease*, 2nd ed. Williams and Wilkins, Baltimore, MD.
- Wildschutte, H., D. M. Wolfe, A. Tamewitz, and J. G. Lawrence. 2004. Protozoan predation, diversifying selection, and the evolution of antigenic diversity in *Salmonella*. *Proc. Natl. Acad. Sci. USA* 101:10644-10649.
- Wilson, M. S., and R. M. Maizels. 2004. Regulation of allergy and autoimmunity in helminth infection. *Clin. Rev. Allergy Immunol.* 26:35-50.
- Yu, V. L., C. C. Chiou, C. Feldman, A. Ortqvist, J. Rello, A. J. Morris, L. M. Baddour, C. M. Luna, D. R. Sydman, M. Ip, W. C. Ko, M. B. Chedid, A. Andremon, and K. P. Klugman. 2003. An international prospective study of pneumococcal bacteremia: correlation with in vitro resistance, antibiotics administered, and clinical outcome. *Clin. Infect. Dis.* 37:230-237.
- Ziegler, E. J., C. J. Fisher, Jr., C. L. Sprung, R. C. Straube, J. C. Sadoff, G. E. Foulke, C. H. Wortel, M. P. Fink, R. P. Dellinger, N. N. Teng, et al. 1991. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N. Engl. J. Med.* 324:429-436.



Color Plate 1. Artist's conception of the infection process and the host's immune response and overresponse: (Blue) site where the presence of bacteria does not result in symptoms—*asymptomatic*. (Red) site or state where the presence of bacteria can result in symptoms. See the text for more details.