

The evolution of bacteria-host interactions: virulence and the immune over-response

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While we blame the bacteria, the morbidity and mortality of most bacterial infections in humans and less well-dressed mammals can be attributed to an inappropriate and/or too-exuberant immune response, an “over-response”. We briefly review the ways the immune system over-responds to infections by different bacteria and the pathology which results. We describe four hypotheses for the evolution of virulence in bacteria and consider how this blame-the-host perspective for the morbidity and mortality of bacterial infections fits these hypotheses for specific diseases and the concept and evolution of virulence determinants. We speculate on why evolution by natural selection has not yet and is unlikely to ever eliminate or even reduce the effects of host over-response responsible for the virulence of many bacterial infections. We conclude with a brief discussion of the implications of the immune over-response to treatment of bacterial infections.

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 much less 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 testable evolutionary hypotheses is not at all that hard for characters that provide obvious fitness advantages to the individual 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 individual bacteria or individual hosts (14), 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 bacteria - 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 bacterial infections can be blamed on the seemingly misguided over-response of the immune defenses, what is sometimes referred to as “friendly fire” (42, 73) or immunopathology (32). 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 (is unable to) blunt the immune over-response 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 over-response:

We define virulence as the magnitude of the morbidity (expression of symptoms) and 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 bacteria–host interaction presented in Figure 1. Bacteria enter a site where they replicate and establish a population, colonize the host, but in which they do not generate perceptible symptoms, the blue box.

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. Penumoniae* bacteremia the blue site is the nasopharynx and the red the blood stream. In this model, the red site needn't be a different physical location. It could also be a different state of the bacteria in the site of their colonization, e.g. for a *Staphylococcus aureus* skin infections the blue site would be the skin and the red a boil.

In this cartoon as well as in mammals, virulence occurs in two ways both of which require the bacteria to enter the red, potentially symptomatic site/state: (i) direct damage to the host tissue due to the replication of the bacteria and/or the production of specific products (toxins) or ii) indirect damage to the host occurs through an over-response of the host 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) Controlling the proliferation of the bacteria in the asymptomatic site
- (3) Controlling 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/state

- (5) Controlling the proliferation of the bacteria within the potentially symptomatic site/state
- (6) Controlling the rate of clearance of bacteria and their products from the potentially symptomatic site/state
- (7) Modulating the immune over-response in the potentially symptomatic site/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/state where they can generate symptoms.

Whether they do generate symptoms and the magnitude of those symptoms given passage into to red site/state also depends on how well the immune system limits their

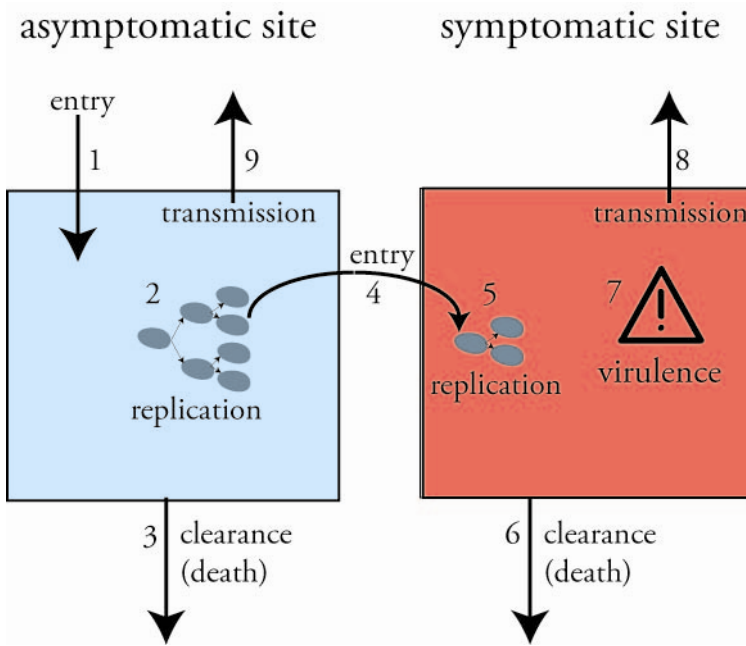


Figure 1. The artist’s conception of the infection process and the host’s immune response and over-response: Blue - site were the presence of bacteria does not result in symptoms – asymptomatic. Red – site/state where the presence of bacteria can result in symptoms. See the text for more details.

densities and the concentrations of their products. In this cartoon, (8) is the infectious transmission of bacteria promoted by the generation of symptoms, and (9) is the transmission of bacteria from the asymptomatic site.

In the following, we focus primarily on the virulence resulting from the over-response 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 include among these are: (i) Dental carries, resulting from the acid produced by metabolizing *Lactobacillus acidophilus* or *Streptococcus mutans* (31) (ii) Paralysis due to the neurotoxins secreted by *Clostridia botulinum* or *Clostridia tetnae* acting on the nerve and motor endplates (67) and (iii) Diarrhea resulting from enterotoxins which inhibit resorption of sodium chloride or promote its secretion. Examples of where virulence is 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 over-response. In this diagram, the immune system is double edged, it can reduce the likelihood of virulence, steps (1)-(6) or by an over-response be the reason for the virulence. Moreover, these two consequences of the immune response may be manifestations of the same process.

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) 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, the cytokines released play an important part in attracting neutrophils (immune cells that phagocytose bacteria) to the local infection site, but is also excessive (38). The distinction between an inappropriate and appropriate immune response can be seen in the spectrum of illness associated with *Mycobacterium leprae* (58, 69) Hosts that respond to infection with predominately antibodies and very few CD4 T cells will have infectious sites with large macrophage that contain numerous mycobacteria these are responsible for the multiple skin lesions and nodules seen in lepromatous leprosy. While a host with T helper 1 type response (high IFN γ production and low IL-4) has numerous well-formed granulomas with very few mycobacteria that

form minor skin lesions. The 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 MHC class II molecules and T cell receptor (beta chain) independently of their specificity for antigens(65). *Staphylococcus 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 which mediate shock and tissue injury. In all three of these cases, the morbidity and mortality of the host can be attributed to an apparently misguided response of the immune system, which for convenience we refer to as an over-response.

Table 1 – Some examples of Virulence resulting from an immune over-response

Disease	Bacteria	Red Site (see figure1)	Bacteria–host interaction (Trigger - Immune overresponse)	Damage induced by immune response
Pneumonia	Multiple species e.g. <i>S. pneumoniae</i> <i>N. meningitides</i> <i>H. influenzae</i>	Lungs	Cell wall and other bacterial components induce pro-inflammatory cytokines, edema and fibrin deposition (7)	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 (55)	Extreme inflammation leading to septic shock
Duodenal Ulcers	<i>Helicobacter pylori</i>	Gastric and duodenal mucosa	Bacterial persistence -chronic inflammation (19)	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 (25)	Tissue necrosis
Petechiae, Waterhouse-friderichsen syndrome	<i>Nisseria meningitides</i>	Skin and adrenal glands	Endotoxin – release of TNF α , IL-1 and IL-6(37)	Blood vessel destruction
Rheumatic Fever	<i>Streptococcuspyrogenes</i>	Heart, Joints, Skin and Brain	M protein- activation of autoreactive (anti-cardiac myosin) antibodies(17)	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 (70)	Inflammation of large joints, eyes and urethra
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Lungs	Bacteria presence- release of high levels of TNF α (4, 36)	Recruitment of fluid and cells into the air spaces of the lungs, necrosis
Meningitis	Multiple species. e.g. <i>S. pneumoniae</i> <i>N. meningitides</i> <i>H. influenzae</i>	Meninges	Cell wall and other bacterial components induce pro-inflammatory cytokines and chemokines (10)	Increased blood-brain barrier permeability, neutrophil recruitment, increased intracranial pressure, brain damage

The evolution of bacterial virulence as an immune over-response:

How does the observation that much of the morbidity and mortality can be attributed to a host over-response to the bacteria help in understanding the evolution of virulence? In a perspective written a decade ago, one of us(40) 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 doubtless biased sample see (3, 9, 11, 12, 20, 21, 29, 33, 48-51, 64)), we don't know of studies that have rejected any of these hypotheses and only one adding what may be a new hypothesis: quasispecies evolution (62). 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 fit each of these hypotheses for the evolution of the virulence of bacteria.

(i) The conventional wisdom: This phrase, which the late John Kenneth Galbraith's coined to describe ideas and explanations that are widely accepted as true by the public, was applied by Bob May and Roy Anderson (54) to describe the then prevailing view of the evolution of the virulence. In 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 populations or both will lead to either 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 feed 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-all-that 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, like *Mycobacteria tuberculosis* are transmitted between humans and can be maintained without the animal source, others like *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” (44), mutualism will prevail.

(ii) 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 life style 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 (41, 47, 53). 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 these epidemiological models.

Regardless of how they are transmitted (directly, by vectors or sexually) the epidemiological fitness (R_0 s) of parasitic bacteria is directly proportional to their rate of

infectious transmission and inversely proportional to the rate at which infected hosts are removed from the transmission pool either by the clearance of the bacteria (entry into an immune or bacteria-free state), the host's death or their removal from the population. For example, if transmission is through contact with respiratory droplets, the maintenance of bacteria in a population of hosts depends on how rapidly it can get from the nasopharynx of one colonized host to a new host. The number of secondary infections also depends on the amount of time the originally colonized host is infectious (which in turn is inversely dependent on the rate at which the bacteria are cleared, the rate the host dies or is otherwise removed from the transmitting pool).

Thus, unless the rate of transmission of the bacteria is proportional to the host's morbidity and mortality ((8) rather than (9) in Figure 1), these epidemiological models can be seen as the theoretical basis of the conventional wisdom (also see (39)). Selection will favor bacteria that are not only infectiously transmitted at ever-higher rates but also persist longer in colonized host (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; host that are less subject to infection-associated morbidity and mortality will be favored. If, however, transmission and the morbidity and mortality of the host are coupled so the more virulent bacteria are transmitted at a 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(20).

On first consideration it may seem the trade-off necessary to maintain virulence in the bacteria 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 it is not. The host over-response could be a byproduct 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 (23)) this interpretation is supported by reasonable plausibility arguments.

Here we considered two of the more compelling of these stories for pathogenic bacteria of humans.

The first are diarrheal diseases for 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 over-response. 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 (35). 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(23), 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 not all that many chromosomal genes (1, 15). 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 in fact suggested that the capacity to generate lethal sepsis is not just a byproduct 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 well 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 over-response is associated with transmission.

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 (46), the arrow above (7) in Figure 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 over-response 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 (22). However, it wasn't until Marshall and Warren (52) presented evidence that a curved bacteria we now known as *H. pylori* was an etiologic agent for gastric and peptic ulcers that this seemingly commensal bacteria was elevated to the status pathogen. This distinction was further enhanced by evidence that *H. pylori* was also associated with gastric cancers (59, 72). *H. pylori* colonization can result in a chronic inflammatory state which 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; virulence is coincidental.

While they are commonly described as pathogens, especially in grant proposals and by people suffering from 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, *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitides*. 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 over-response in these sites (7, 10). The occasional movement of bacteria into site where they can cause disease, the red in Figure 1, may be due to chance/coincidental evolution or as we argue below may be a consequence of within-host evolution of the bacterial population.

iv) 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 (43). The advantage gained by the bacteria by generating symptoms in a colonized host is not to its advantage beyond that particular 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 where 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, like the polio and coxsackievirus, being the product of within-host evolution (30, 43), for bacteria the best we can do at this stage are arguments founded on plausibility and consistency with observations, see for example (56). 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 (57, 60, 63, 66). One interpretation of 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 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 Figure 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 bacteria is coincidental or due to within-host evolution, the virulence of bacteria in these sites can be attributed to the host's immune over-response.

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 are 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 (24). Included among these are characters that facilitate adhesion to host cells, evade the host constitutive and inducible immune defenses and produce toxins. And, appropriately much of contemporary bacteriology is devoted to understanding the molecular biology, genetics, evolutionary origin and mode of action virulence determinants as a way to understand bacterial diseases and ideally prevent or treat them.

While virulence determinants are almost certainly the products of adaptive evolution in bacterial populations, not so clear are the selection pressure responsible for their evolution and maintenance. Are they favored because of virulence, e.g. the morbidity and mortality of the host promotes the colonization, persistence and infectious transmission of bacteria that express these determinants? Are they byproducts of selection for other functions, e.g. their expression provides protection against grazing

protozoa e.g.(74) and/or facilitate competition with other microbes? Or is the virulence attributed to these factors an inadvertent byproduct 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 bacteria-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 over-response.

Why does the immune system over-respond? In the preceding, we have portrayed the host immune system as misguided, over-responding 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 over-responding 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 (32). Here we offer our perspective on this issue (overlapping somewhat, but not entirely with that of Graham and colleagues).

As we see it, there are two general classes of explanations for the maintenance of an over-response of the immune system: (i) While infectious disease may be a major source of morbidity and mortality (34), 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) There are functional constraints on the immune system which limit the ability of natural selection to totally prevent and maybe even partially mitigate an immune over-response to bacterial infections.

(i) Even if selection universally favors tempering the immune over-response to infections, and the favored genotypes could be generated (which we question below, ii) the time required for temperance to evolve could be considerable, especially if the over-response is specific for particular bacteria and/or their products. This is due to two factors: (a) At

the maximum the intensity of selection for modulating the immune over-response 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, are rarely lethal or sterilizing, are primarily manifest after reproductive years, or the magnitude of the reduction of the over-response of the favored genotype is 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 over-response 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 over-response was 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 1381 generations (more than 20,000 years for humans) for that gene to reach a gene frequency of 50%. If the favored genotype is a recessive the corresponding number of generations would be 100,491(16).

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 mammal hosts. We suggest, however, that this edge contributes little if anything to the slowing pace at which mammalian evolution could modulate the immune over-response. Although there are situations where virulence is positively correlated with the infectious transmission of bacteria (the second hypothesis for the evolution of virulence see page) 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 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 the evolution of a more temperate host immune system. Of all the examples considered in this chapter, the only one where evolution in the bacterial

population might favor an immune over-response is Elisabeth's Carneil's suggestion that by killing their host, *Yersinia 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 systems over-response 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 over-response to bacterial and other infections are functional constraints which limit the extent to which the immune system can be modified. The immune system has roles other than the clearing bacteria infections. It has in fact been postulated that these other roles dominated the evolution of the mammalian immune system (13). 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 over-response of those elements of the immune system that in less-pristine times would otherwise be occupied with the control of helminth infections (75).

There is a fine line between responding (1-6 in Figure 1) and over-responding (7 in that figure), which may be difficult for the systems regulating the immune response to perceive much less avoid. As suggested by Steve Frank (2) 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 over response.

Is there evidence in support of these two hypotheses for why evolution has not eliminated the virulence resulting from the immune over-response? 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 (5, 6, 68, 71) 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, 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 the myopia and limitations of natural selection; (i) the relative weakness of selection for modulating the immune over response 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 (16).

Implications: While the morbidity and mortality of most bacterial infections can be attributed to an immune over-response, 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. Treatment commonly fails, patients die or remain ill for extensive periods. Resistance of the pathogen to the antibiotics employed for treatment is only one of the reason for this failure and for some infections is not the major one, at least not yet (45, 76).

The obvious alternative approach to treating infections is to reduce the morbidity and prevent the mortality by modulating the immune system's over-response. There have been attempts to do just that for the treatment of bacteria-mediated sepsis. Clinical trials have evaluated the use of glucocorticoids(8) and drugs designed to neutralize endotoxins (77), tumor necrosis factor α (26) or interleukin-1 β (27) but none of these treatments were effective. The most successful trials in humans to date has 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 clots formation which are responsible for organ failure seen in late stages of sepsis) (28). In addition, there are new agents which are directed at redirecting the immune response and hold promise as effective future therapies for sepsis, such as IL-12(61) or antibodies against complement (C5a) (18). However understanding the specifics of the immune over-reaction 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.

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References

1. **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 U S A* 96:14043-8.**
2. **Andre, J.-B., S. Gupta, S. Frank, and M. Tibayrenc. 2004. Evolution and Immunology of infectious diseases: what new? An E-debate. *Infection Genetics and Evolution* 4:69-75.**
3. **Andre, J. B., and B. Godelle. 2006. Within-host evolution and virulence in microparasites. *J Theor Biol* 241:402-9.**
4. **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* 68:6954-61.**
5. **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 U S A* 97:8005-9.**
6. **Bellamy, R., and A. V. Hill. 1998. Genetic susceptibility to mycobacteria and other infectious pathogens in humans. *Curr Opin Immunol* 10:483-7.**
7. **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* 66:912-22.**
8. **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* 92:1032-6.**
9. **Bonhoeffer, S. A., and M. A. Nowak. 1994. Mutation and the evolution of virulence. *Proceedings of the Royal Society London, B.* 258:133-140.**
10. **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.**
11. **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.**
12. **Bull, J. J. 1994. Virulence. *Evolution* 48:1423-1437.**
13. **Burnet, F. 1970. *Immunological Surveillance*.**
14. **Burnet, F. M., and D. O. White. 1972. *Natural history of infectious diseases*. Cambridge university press.**

15. Carniel, E. 2003. Evolution of pathogenic *Yersinia*: some lights in the dark p. 3-12, *Advances in Experimental Medicine*, vol. 529. Springer Verlag.
16. Crow, J. F., and M. Kimura. 1971. *An Introduction to Population Genetics Theory*, First ed. Harper Row, New York.
17. Cunningham, M. W. 2003. Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. *Front Biosci* 8:s533-43.
18. 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* 5:788-92.
19. Czinn, S. J., and J. G. Nedrud. 1997. Immunopathology of *Helicobacter pylori* infection and disease. *Springer Semin Immunopathol* 18:495-513.
20. 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.
21. Ebert, D., and E. A. Herre. 1996. The evolution of parasitic diseases. *Parasitol Today* 12:96-101.
22. 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. Katzowitsch, X. Wang, M. Achtman, and S. Suerbaum. 2003. Traces of human migrations in *Helicobacter pylori* populations. *Science* 299:1582-5.
23. Fenner, F., and F. N. Ratcliffe. 1965. *Myxomatosis*. Cambridge university press, Cambridge.
24. Finlay, B. B., and S. Falkow. 1989. Common themes in microbial pathogenicity. *Microbiol Rev* 53:210-30.
25. 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* 167:1309-20.
26. 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* 334:1697-702.
27. 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.
28. Fourrier, F. 2004. Recombinant human activated protein C in the treatment of severe sepsis: an evidence-based review. *Crit Care Med* 32:S534-41.

29. Frank, S. A. 1996. Models of parasite virulence. *Quarterly Review of Biology* In Press.
30. 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 U S A* 103:13825-30.
31. Gibbons, R. J. 1964. Bacteriology of Dental Caries. *J Dent Res* 43:SUPPL:1021-8.
32. Graham, A. L., J. E. Allan, and A. F. Read. 2005. Evolutionary causes and consequences of immunopathology. *Annual Review of Ecology, Evolution and Systematics* 36:373-397.
33. Grech, K., K. Watt, and A. F. Read. 2006. Host-parasite interactions for virulence and resistance in a malaria model system. *J Evol Biol* 19:1620-30.
34. Haldane, J. B. S. 1949. Disease and evolution. *La Ricerca Scientifica* 19:68-76.
35. Hilbi, H., A. Zychlinsky, and P. J. Sansonetti. 1997. Macrophage apoptosis in microbial infections. *Parasitology* 115 Suppl:S79-87.
36. 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 U S A* 99:8330-5.
37. Klein, N. J., C. A. Ison, M. Peakman, M. Levin, S. Hammerschmidt, 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* 173:172-9.
38. 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* 104:743-50.
39. Lenski, R. E., and R. M. May. 1994. The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *Journal of Theoretical Biology* 169:253-65.
40. Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. *Emerging Infectious Diseases* 2:93-102.
41. Levin, B. R., A. C. Allison, H. J. Bremermann, -, S. Cavalli, L.L., B. C. Clarke, R. Frenzel-Beymem, W. D. Hamilton, S. A. Levin, R. M. May, and H. R. Thieme. 1982. Evolution of parasites and hosts (group report), p. 212-243. *In* R. M. Anderson, R. M. May, and D. conf. (ed.), *Population Biology of Infections Diseases*. Springer, Berlin.
42. Levin, B. R., and R. Antia. 2001. Why we don't get sick: the within-host population dynamics of bacterial infections. *Science* 292:1112-5.

43. Levin, B. R., and J. J. Bull. 1994. Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends in Microbiology* 2:76-81.
44. Levin, B. R., V. Perrot, and N. Walker. 2000. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* 154:985-97.
45. Levin, B. R., and D. E. Rozen. 2006. Non-inherited antibiotic resistance. *Nat Rev Microbiol* 4:556-62.
46. Levin, B. R., and C. Svanborg Eden. 1990. Selection and evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* 100:S103-15.
47. Levin, S. A., and D. Pimentel. 1981. Selection of intermediate rates of increase in parasite host systems. *American Naturalist* 117:308-315.
48. 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.
49. Lipsitch, M., and E. R. Moxon. 1997. Virulence and transmissibility of pathogens: what is the relationship? *Trends in Microbiology* 5:31-7.
50. Lipsitch, M., S. Siller, and M. A. Nowak. 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* (In Press).
51. Mackinnon, M. J., and A. F. Read. 2004. Virulence in malaria: an evolutionary viewpoint. *Philos Trans R Soc Lond B Biol Sci* 359:965-86.
52. Marshall, B. J., and J. R. Warren. 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1:1311-5.
53. May, R. M., and R. M. Anderson. 1983. Epidemiology and genetics in the coevolution of parasite and hosts. *Proceedings of the Royal Society, London, Series B* 219 B:281-313.
54. May, R. M., and R. M. Anderson. 1983. Parasite - host coevolution, p. 186-206. *In* D. J. Futuyama and M. Slatkin (ed.), *Coevolution*. Sinauer, Sunderland Massachusetts.
55. 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.
56. 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* 270:1667-77.
57. 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.

58. Modlin, R. L. 2002. Learning from leprosy: insights into contemporary immunology from an ancient disease. *Skin Pharmacol Appl Skin Physiol* 15:1-6.
59. Moss, S. F., and M. J. Blaser. 2005. Mechanisms of disease: Inflammation and the origins of cancer. *Nat Clin Pract Oncol* 2:90-7; quiz 1 p following 113.
60. Moxon, E. R., and P. A. Murphy. 1978. *Haemophilus influenzae* bacteremia and meningitis resulting from the survival of a single organism. *Proceedings of the National Academy of Sciences, USA* 75:1534-1536.
61. 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* 120:290-6.
62. Pfeiffer, J. K., and K. Kirkegaard. 2005. Increased fidelity reduces poliovirus fitness and virulence under selective pressure in mice. *PLoS Pathog* 1:e11.
63. 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.
64. Regoes, R. R., M. A. Nowak, and S. Bonhoeffer. 2000. Evolution of virulence in a heterogeneous host population. *Evolution Int J Org Evolution* 54:64-71.
65. Rott, O., and B. Fleischer. 1994. A superantigen as virulence factor in an acute bacterial infection. *J Infect Dis* 169:1142-6.
66. Rubin, L. G. 1987. Bacterial colonization and infection resulting from multiplication of a single organism. *Rev Infect Dis* 9:488-93.
67. 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* 359:832-5.
68. Segal, S., and A. V. Hill. 2003. Genetic susceptibility to infectious disease. *Trends Microbiol* 11:445-8.
69. 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* 162:1851-8.
70. Sieper, J. 2001. Pathogenesis of reactive arthritis. *Curr Rheumatol Rep* 3:412-8.
71. Sorensen, T. I., G. Nielson, P. Anderson, and T. Teasdale. 1988. Genetic and environmental influences on premature death in adult adoptees. *New England Journal of Medicine* 318:727-732.

72. **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.**
73. **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.**
74. **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 U S A* 101:10644-9.**
75. **Wilson, M. S., and R. M. Maizels. 2004. Regulation of allergy and autoimmunity in helminth infection. *Clin Rev Allergy Immunol* 26:35-50.**
76. **Yu, V. L., C. C. Chiou, C. Feldman, A. Ortqvist, J. Rello, A. J. Morris, L. M. Baddour, C. M. Luna, D. R. Snyderman, M. Ip, W. C. Ko, M. B. Chedid, A. Andremont, 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* 37:230-7.**
77. **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, and 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* 324:429-36.**