CHAPTER 11

The Damage-Response Framework of Microbial Pathogenesis and Infectious Diseases

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Abstract

Historical and most currently held views of microbial pathogenesis and virulence are plagued by confusing and imprecise terminology and definitions that require revision and exceptions to accommodate new basic science and clinical information about microbes and infectious diseases. These views are also inherently unable to account for the ability of some microbes to cause disease in certain, but not other hosts, because they are grounded in singular, either microbe-or host-centric views. The damage-response framework is an integrated theory of microbial pathogenesis that puts forth the view that microbial pathogenesis reflects the outcome of an interaction between a host and a microbe, with each entity contributing to the nature of the outcome, which in turn depends on the amount of host damage that results from the host-microbe interaction. This view is able to accommodate new information and explain why infection with the same microbe can have different outcomes in different hosts. This chapter describes the origins and conceptual underpinnings of and the outcomes of infection put forth in, the damage-response framework.

Introduction to the Damage-Response Framework

The damage-response framework is a theory of microbial pathogenesis that was first proposed in 1999 in an effort to account for the contribution of both the host and the microbe in microbial virulence and pathogenicity.[1] Until that time concepts of microbial pathogenesis were largely microbe-or host-centric, in that they attempted to explain microbial virulence in the context of microbial properties or host susceptibility, respectively. Microbe-centric views regard virulence and pathogenicity as singular microbial traits, e.g., as the result of the action of a microbial factor or determinant that injures the host. Host-centric views regard virulence and pathogenicity as host-dependent outcomes that result from a defect or deficiency in the host. In contrast, the damage-response framework is neither microbe-nor host-centric but focuses on the outcome of the host-microbe interaction and emphasizes that host damage is the common denominator that is relevant to any host-microbe interaction. The damage-response framework reconciles microbe-and host-centric views by incorporating the recognition that both the microbe and the host contribute to pathogenicity and virulence. It is based on three tenets that are considered to be both obvious and incontrovertible: (1) that microbial pathogenesis requires two entities, a host and a microbe

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and that the two entities must interact; (2) that the host relevant outcome of host-microbe interaction is damage to the host; (3) host damage can occur as a result of microbial factors, host factors, or both.[2] These tenets are represented graphically by the basic damage-response curve, a U shaped curve that depicts host damage on the Y axis as a function of the host immune response, which is depicted from weak to strong along the X axis. The U shape of this curve illustrates that host damage can be maximal in the setting of a weak or a strong host response (Fig. 1).

Conceptual Origin of the Damage-Response Framework

The damage-response framework originated as a teaching tool in the graduate microbial pathogenesis course at the Albert Einstein College of Medicine in the mid-1990s. While teaching we found it very difficult to convey to students the concept that some microbes were pathogenic only in certain hosts using the then existing treatises on pathogenicity and virulence. The inability of either microbe-centric or host-centric views to account for the late 20th century emergence of diseases caused by microbes previously considered to be nonpathogens and the emergence of the diseases caused by these microbes in individuals with immune impairment was the catalyst for proposing a different approach to the problem.[3] These microbes included *Candida albicans* and *Staphylococcus epidermidis*, which emerged as leading causes of bloodstream infections when they had long been held to be nonpathogens.[4,5] The late 20th century witnessed an unprecedented increase in individuals with immune impairment due to predominantly 4 factors: (1) the use of plastic catheters to deliver intravenous fluids and medications in the hospital setting; (2) the rise in antibiotic use, overuse and misuse;[6] (3) the development and use of immunosuppressive therapies for malignancy and to combat organ rejection in the setting of organ transplantation; and (4) the HIV/AIDS pandemic (Fig. 2). Each of these factors led to the emergence of distinct populations of individuals with impaired immunity and it was among these individuals that many microbes previously considered to be nonpathogens were associated with disease. The observation that microbes previously considered to be nonpathogenic could be pathogens led to the concept of microbial 'opportunism',[7] an unfortunate term that introduced the anthropomorphic view that these microbes were somehow taking advantage of the host to cause disease. In fact, many of the pathogens labeled as opportunistic were components of the normal microbial flora, such as *Candida albicans* and *Staphylococcus epidermidis*. The convergent emergence of diseases caused by microbes long held to be nonpathogens and newly emergent populations of immunocompromised individuals brought to the fore that infectious diseases can only occur in susceptible individuals. Although the veracity of this statement is immediately obvious, this notion is distinctly absent in microbe-centric views which regard microbial virulence as a microbial property. The veracity of this statement is further underscored by the fact that diseases caused by vaccine-preventable microbes, eg smallpox, do not occur in immune individuals and that the clinical manifestations of infectious diseases often reflect the host inflammatory response, in some cases, even in the absence of the causative microbe. These points, which are largely agreed upon by the infectious diseases and microbial pathogenesis fields, issue a serious challenge to prevailing definitions of pathogenicity and virulence, since the same microbe could be either a pathogen or nonpathogen, depending on the host.

The Lexicon of the Damage-Response Framework

A central feature of the Damage-response framework is a simple, self explanatory lexicon that does not require exceptions or corollaries to define the components of microbial pathogenesis and virulence. The key to understanding the lexicon is that according to the damage-response framework, the essential components of microbial pathogenesis reduce to two entities, hosts and microbes and the damage that occurs in the host as a result of their interaction. Furthermore, the outcome of the interaction can change as a function of time depending on the amount of damage that occurs in the host. The damage-response framework does not view pathogens and nonpathogens as intrinsically different; based on incontrovertible evidence that the same microbe can be a pathogen or nonpathogen, depending on the host. Hence, the terms 'pathogen' and 'nonpathogen' only have

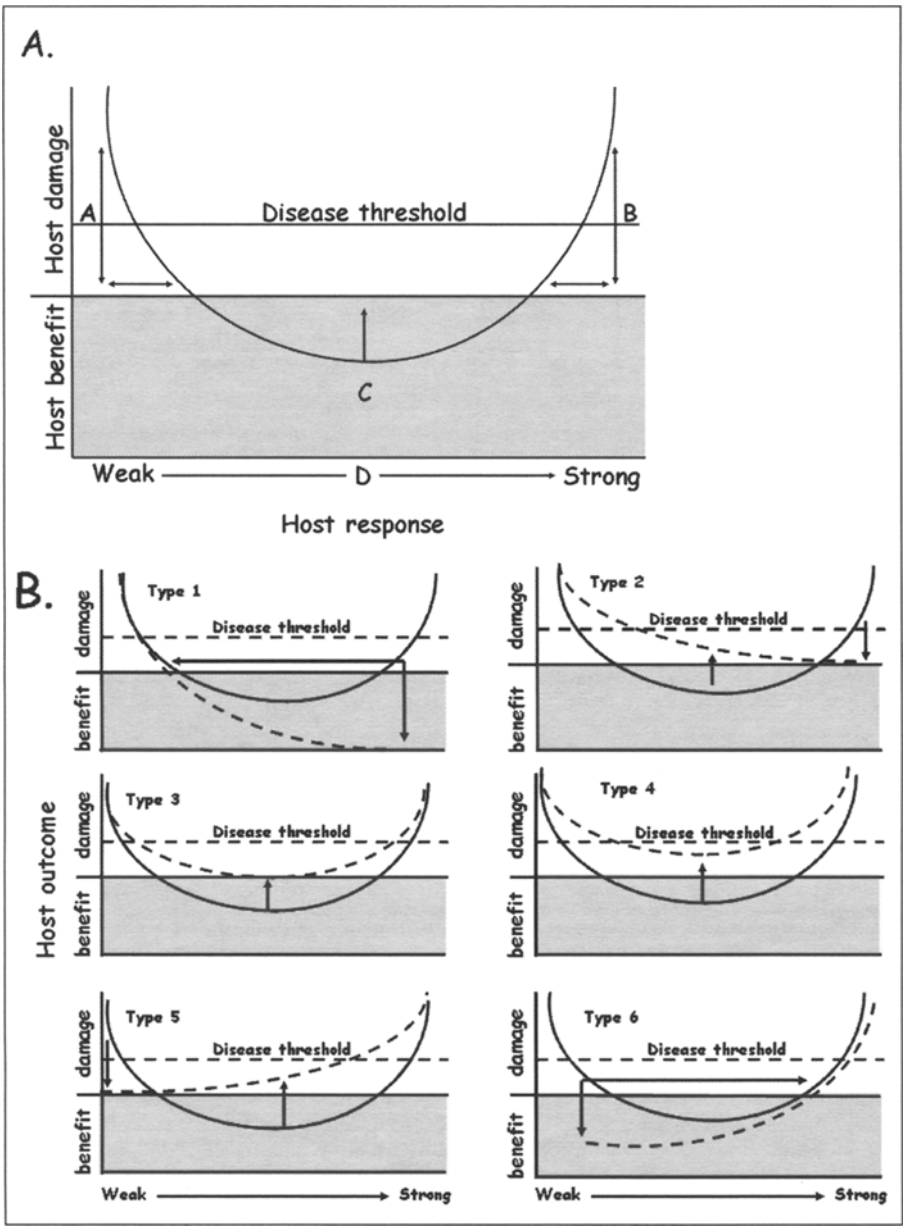

Figure 1. The damage response curve. Host damage is depicted as a function of the host response along a continuum from weak to strong. A) The solid U shaped curve demonstrates that certain host- microbe interactions confer a host benefit. The arrow (C) illustrates that the curve can shift upwards. The arrows at each side of the curve (A, B) illustrate that the curve can shift downward and to the left and right. B) Damage-response curves that reflect the outcome of different host- microbe interaction can be derived from the basic curve. Examples of microbes that result in these types of curves are as follows: Type 1—*Staphylococcus epidermidis*; Type 2—Hepatitis A virus; Type 3—*Aspergillus spp.*; Type 4—*Histoplasma capsulatum*; Type 5—SARS coronavirus; Type 6—*Helicobacter pylori*.

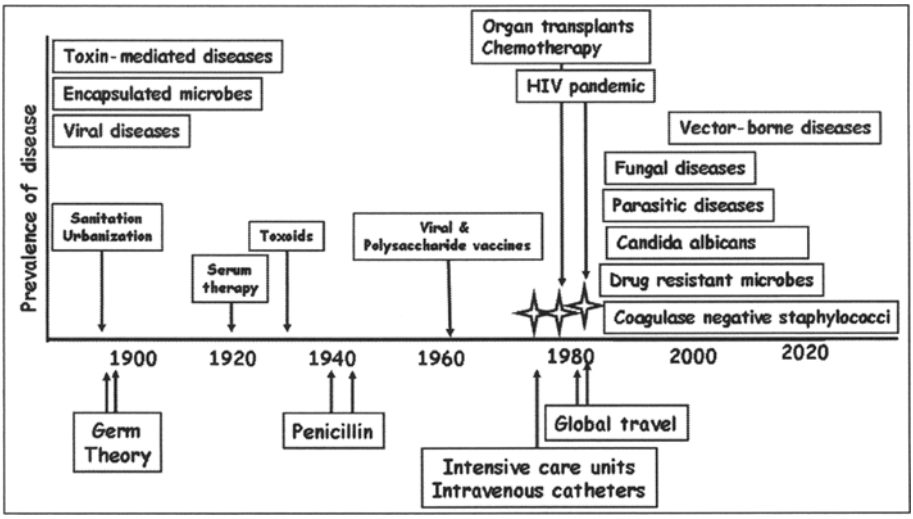

Figure 2. The spectrum of infectious diseases 1900-2000. The prevalence of disease due to the indicated microbes is shown on the Y axis as a function of time and the innovations and factors depicted on the X axis.

meaning in the context of a given host. The damage-response framework defines a pathogen as a microbe with the potential to cause damage in a host.[8] This definition avoids linking the nature of a pathogen to mechanisms by which it causes disease and encompasses microbial diversity, which extends from microbes that invade host cells to those that do not, or are macroscopic, such as *Shigella sp* and *Vibrio cholera* and *Ascaris lumbroides*, respectively, to those that have a normal niche, such as *Candida albicans* and *Staphylococcus epidermidis*, to those that are encoded by the host, such as prions. Virulence is defined as the relative capacity of a microbe to cause damage in a host.[9] The term 'relative' is necessitated by the fact that, at present, damage cannot be fully quantified; because precise readouts of host damage remain limited and available tools and platforms are insufficient for quantification. Furthermore, virulence has been and continues to be a relative term since any measurement of virulence is relative to a control condition or strain. Despite this gap, there is little difficulty in identifying or agreeing upon currently available readouts of host damage. When host damage surpasses a threshold that maintains host homeostasis, clinical disease occurs. With these definitions, the Damage-response framework dispenses with imprecise and confusing terms, such as nonpathogen, partial pathogen, primary pathogen, opportunistic pathogen, commensal and saphyrophyte. The problems of imprecise and shifting terminology are immediately apparent when one considers a microbe such as *Candida albicans* which is considered a commensal in most hosts, an opportunistic pathogen in patients with impaired immunity and even a primary pathogen in women with no obvious immune deficit that suffer from candidal vaginitis. The damage-response framework defines the term infection as the acquisition of a microbe, rather than to describe an illness or condition. This enables a more precise understanding of microbial pathogenesis that is consistent with the fact that infection with a microbe is not synonymous with it causing damage or disease.

The Damage Response Curve

The U shaped damage-response curve illustrates the complex origins of host damage by depicting it on the Y axis as a function of the host response along a continuum from weak to strong on the X axis (Fig. 1). The curve is U shaped, because host damage can occur in the setting of either a weak or a strong host response. The host response encompasses the full range of host immunity such that

weak and strong responses lack essential components that are required for the normal, or appropriate, response, which results in a minimum amount of damage, most likely due to counterbalancing responses. For example, the response to a microbe often produces an initial inflammatory response, which is later counterbalanced by a dampening of the response. The absence of an appropriate initial or a counterbalancing response can each result in host damage. Damage in the setting of a weak host response often reflects microbe-mediated damage, such as that caused by the action of microbial factors and damage in the setting of a strong host response often reflects host-mediated damage, such as that caused by excessive inflammation. Microbial factors that cause host damage include capsular polysaccharides, toxins, proteases and components that are toxic to host cells. Most of these factors cause more damage in the setting of weak responses. Host factors that cause host damage include immune complexes, cytokines, chemokines and microbicidal peptides. The recognition that host damage can occur at the extremes of the host response underscores that the outcome of microbial infection is an interaction, whereby singular host responses are insufficient to prevent or minimize host damage and an interplay that achieves a balanced response is most successful at damage control. The damage-response curve is inherently flexible and can be used to plot any host-microbe interaction.

The States of Infection

In addition to depicting host damage as a function of the host response, the damage-response framework also depicts host damage as a function of time. Hence, damage is a function of the host response at a given time (Fig. 1) and damage is a function of time for a given host response (Figs. 3, 4). According to this schema, there are 5 outcomes of microbial infection: elimination; colonization, commensalism, disease and latency (Fig. 3).[3,10] Colonization, commensalism, disease and latency are distinguishable by the amount of host damage over time. Changes between these states occur, usually as a result of a change in the host immune response (Figs. 3, 4).

Colonization is a state in which the amount of host damage is potentially measurable, but less than the disease threshold.[10] Although the methodology for measuring damage in states of colonization does not currently exist we note that this state is often associated with the development of an immune response which may reflect the occurrence of some degree of damage that is less than that which translates into disease. For most microbes, colonization is a transient state,

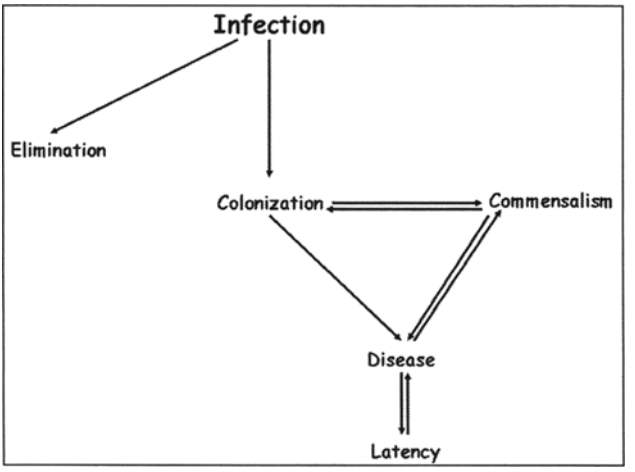

Figure 3. The five outcomes of microbial infection. The interrelationships between colonization, commensalism, disease and latency are depicted by arrows between the relevant outcomes. Factors that induce change from one state to another include immunosuppression, reduced barrier immunity (due to the insertion of vascular catheters), cytotoxic agents and therapy.

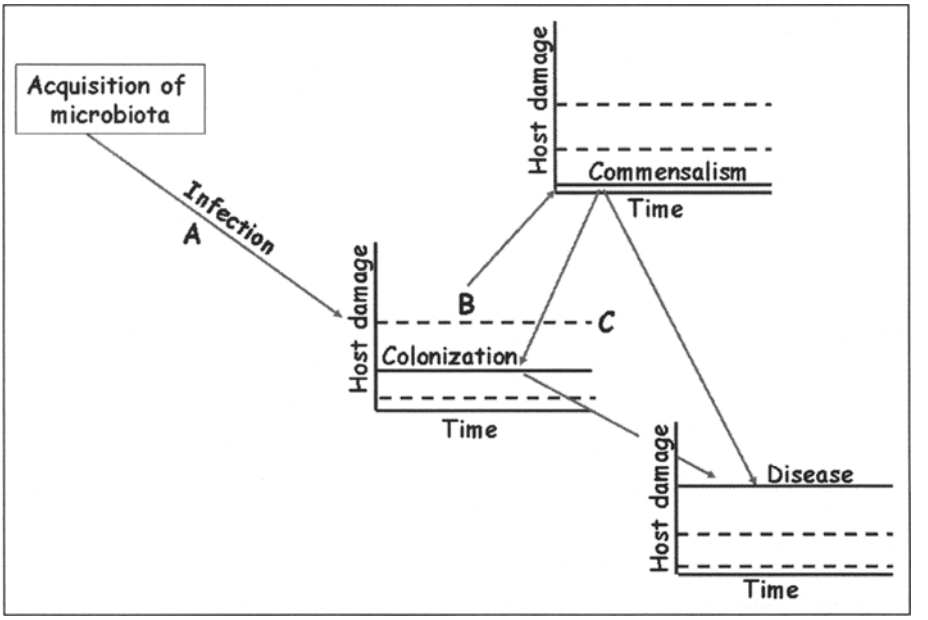

Figure 4. The acquisition of the microbiota and transitions between commensalism, colonization and disease. The relevant state is depicted by a solid line, the possible transition states are depicted by dashed lines. The transitions from infection to colonization (A) and from colonization to commensalism (B) occur early in life. The transition from commensalism to colonization or to disease, directly or indirectly, can occur when the microbiota is disrupted or eliminated due to invasion of skin or mucosal surfaces with catheters or surgery, antibiotic or cytotoxic therapy, or immunosuppression, or when host factors compromise its functioning (C).

during which the microbe can be isolated from the host and may be evidence of a host immune response. The types of host responses or damage that accompany colonization include serological evidence of infection, cellular responses that result in tissue responses, such as granulomas or giant cells and immune responses that result in inflammation and cellular recruitment. Whether the stimulus for such immune responses is microbe-mediated damage is uncertain at this time. The state of colonization can lead to elimination or transition to commensalism or disease. Elimination can result from immune mechanisms, e.g., by an immune response to a respiratory microbe, such as *Streptococcus pneumoniae*, or intervention, such as antimicrobial agents. Colonization transitions to disease when the amount of damage exceeds the disease threshold. This occurs when host mechanisms or intervention fail to limit host damage. The failure of host mechanisms often reflects weak or inappropriate immune responses, such as those that predispose individuals with antibody and B-cell defects to disease with *Streptococcus pneumoniae*, or individuals with defects in cellular immunity to disease with *Cryptococcus neoformans*. Colonization changes to commensalism following microbial acquisition soon after birth.

Commensalism is a unique state in which host-microbe interaction that either provides a host benefit or no outcome, rather than resulting in host damage.[10] There is no host damage in the state of commensalism. The state of colonization becomes indistinguishable from commensalism when the amount of host damage attributable to colonization is negligible. Hence, *Staphylococcus aureus* in the nares of a chronic asymptomatic carrier may be indistinguishable from a commensal microbe, with the caveat that in aggregate, some of the microbes that assume the state of commensalism impart a host benefit. The host is generally defined as the entity that microbes inhabit. However, the number of microbes that inhabit the human body exceeds the number of human

cells, calling into question the definition of host. The gastrointestinal tract is inhabited by more than 10^{13} microbes, with more than 100 times the number of genes as the human genome.[11] Hence, the state of commensalism provides a host habitat for vast and complex microbial communities. These communities collectively referred to as the microbiota include microbes originally thought to be acquired soon after birth. However, an emerging body of evidence suggests that the human microbiota is even more diverse than previously suspected and influenced by a myriad of host factors.[12] The diversity amongst and the regulatory and immunomodulatory roles that the human microbiota play has only recently begun to be unraveled,[11] principally through the use of innovative techniques that allow for the identification of unculturable microbes.[13] In addition to unculturable microbes, scores of culturable Gram negative and Gram positive, anaerobic and aerobic bacteria and *Candida albicans* inhabit the human host. The acquisition of these microbes can be associated with damage and disease, such as necrotizing enterocolitis and disseminated candidiasis in infants. However, in most instances, the acquisition of these and other microbes is not associated with disease. Microbes that inhabit the gastrointestinal tract are thought to contribute to the development and maintenance of natural immunity.[14-16] Although the microbial determinants and mechanisms that stimulate immunity remain to be fully understood, the importance of the microbiota for normal immunity is supported by evidence that host damage ensues when there is a failure to acquire or disruption of the microbiota. When this occurs, there is a transition from the state of commensalism to the state of colonization or of disease. The microbiota can be disrupted by surgical intervention, antimicrobial therapy, cytotoxic agents and radiotherapy. In addition to contributing to natural immunity, the microbiota play an important role in maintaining the integrity of host tissues, through the elaboration of protective substances and via colonization resistance, including mechanisms resulting in inhibition of other microbes with a greater potential to induce damage from gaining access to host receptors and tissues.

Disease is a state where host damage exceeds the threshold for clinical symptoms. The state of disease can change to elimination with intervention or if host immune mechanisms are sufficient to reduce the amount of damage to below the disease threshold. An inability to reduce damage below the disease threshold can reflect a failure of host immune mechanisms or an intervention to eliminate a microbe or control damage, or both. Interventions for infectious diseases endeavor to treat or prevent the state of disease. Most available interventions focus on microbial elimination, but such therapies often do not control the host response, because the state of disease often reflects aspects of the host response that induce inflammation and enhance the inflammatory response. The state of disease can change to latency, a state in which the microbe remains in the host and vital, but induces damage that is below the disease threshold. The inability to reduce damage in the state of disease ultimately results in chronic disease or death.

Latency is a state that is characterized by a microbial presence, whereby survival of the microbe produces an amount of damage that is below the disease threshold.[10] Latency does not have an obvious host benefit although it is conceivable that changes to the immune system by continued stimulation with microbial antigens forestalls the development of other conditions, such as allergic diseases (e.g., 'hygiene hypothesis').[17] For example, helminth infections have been associated with protection against the development of asthma[18] and patients with positive tuberculin reactions indicative of latent *Mycobacterium tuberculosis* infection had reduced atopy.[19] Latency is a state in which a microbe survives in host cells in a manner that prevents it from elimination, often due to factors that allow it to escape host immune surveillance. Mechanisms that enable latency include the capacity for intracellular survival and persistence, such as for Herpes and other viruses, the induction of tissue responses that contain and control growth of the microbe, such as granulomas for Mycobacteria and fungi and residence in sequestered sites, such as for HIV. The state of latency can transition to disease with a change in the immune status of the host. Major risk factors for this transition are diseases and interventions that impair host immunity, such as immunosuppressive agents given for malignancy, inflammatory diseases and stem and organ transplantation and HIV.

In summary, the outcomes of host-microbe interaction result in 4 states, which differ only in the amount of host damage, or benefit. The states are not fixed by the microbe, but by the amount

of damage that ensues from a host-microbe relationship. Since the outcome of a host-microbe interaction depends on host and microbial factors, knowledge of the nature of the host immune response, host immune status and microbial factors makes it possible to predict the likely state for a given host and microbe.

The Utility of the Damage-Response Framework

The utility of the Damage-response framework is reflected in its flexibility, ability to incorporate new information and explain previous information that could not be accounted for by other views of pathogenesis and virulence. For example, the damage-response framework is able to account for why previously rare diseases, such as those caused by *Cryptococcosis neoformans* and *Pneumocystis pneumonia* occurred in epidemic proportions in individuals with HIV infection. Similarly, the damage-response framework is able to account for the emergence of *Candida albicans* as a major human pathogen in immunocompromised hosts. In addition to accounting for diseases in weak hosts, the damage-response framework can also account for diseases with excessive host responses, such as toxic shock syndrome, Kawasaki disease, allergic aspergillosis and mediastinal fibrosis. An important corollary of the damage-response framework is that infectious diseases can only occur in susceptible hosts. This concept is central to understanding whether the outcome of host-microbe interaction results in host damage, is neutral or beneficial.

Applications of the Damage-Response Framework

Education

The damage-response framework has proven to be a useful educational tool for teaching microbial pathogenesis, infectious diseases, microbiology and immunology to graduate and medical students. The advantages of teaching these disciplines based on a theoretical construct is that it leads to the use of a more universal lexicon, which enhances communication and sharpens the rigor and sophistication of research questions.

Determining the Weapon Potential of a Microbe

The lists used to categorize potential microbe-based weapons lack grounding in principles of microbial pathogenesis. The concepts of pathogenicity and virulence put forth in the damage-response framework were used to derive a standardized formula to determine the weapon potential of microbes based on the transmissibility of the microbe, the inoculum required to cause disease and the time to disease.[20,21] This formula provides a rationally based approach to assessing the potential threat that a microbe could pose as a biological weapon. In view of the corollary of the damage-response framework that infectious diseases can only occur in susceptible hosts, the damage-response framework-based formula for weapon potential provides a strategy for counteracting the threat of microbial agents of bioterror based on bolstering host immunity.

Providing Guidance on the Development of New Therapies

The Damage-response framework provides a conceptual basis for the development of new approaches to preventing and treating infectious diseases.[22,23] The functional outcome of therapies for infectious diseases is that they prevent or ameliorate the host damage that results in the state of disease. Some diseases are caused by microbe-mediated damage, while others are caused by host-mediated damage and others may result from damage due to the lack of microbially produced factors. Some diseases cannot be treated in hosts with impaired immunity and treatment of some diseases with antimicrobial agents fails to ameliorate host damage. The recognition that host damage can occur at the extremes of the host response issues a challenge to the development of therapeutics, since approaches to counteracting the damage caused by microbial factors are inherently different than approaches to counteracting damage caused by host factors. Treatment of damage due to microbial factors requires a focus on enhancing the ability of the host to eliminate the microbe or neutralize its components, whereas treatment of damage due to host factors requires a focus on reducing the inflammatory response. Each of these conditions lies on a different part of the damage-response

curve (Fig. 5). As such, intervention for a patient with damage caused by an insufficient response could require enhancement of the host response with adjuvants, cytokines or immunostimulants. In contrast, intervention for a patient with damage caused by an excessive response could require reducing the host response with steroids, immunomodulators or immunosuppressive agents. The dichotomous origins of host damage in microbial pathogenesis and infectious diseases provide the basis for a rational approach to the use of immunotherapeutic agents for infectious diseases.

Revealing New Paradigms in Host Immunity

The Damage-response framework was used to re-examine the long held view that immunity to intracellular microbes is mediated by the cellular arm of the immune system and immunity to extracellular microbes is mediated by the humoral/antibody arm.[24] A new view was put forth that antibody immunity can confer protection against a myriad of intracellular and extracellular microbes by classical and novel mechanisms that promote damage control.

Understanding the Role of the Host Microbiota in Health and Disease

Given that the damage-response framework does not view microbes as inherently pathogenic or nonpathogenic, it views the complex microbiota associated with the human host in the context of the outcome of their interaction. Hence the interaction between a healthy host and the host-associated microbiota is essential for the normal development of the immune system and for host nutrition and homeostasis. In health, the host-associated microbiota also provides a central layer of host defense by occupying a niche and preventing other microbes from establishing themselves. This community interacts with the immune system and may be regulated by immune responses to

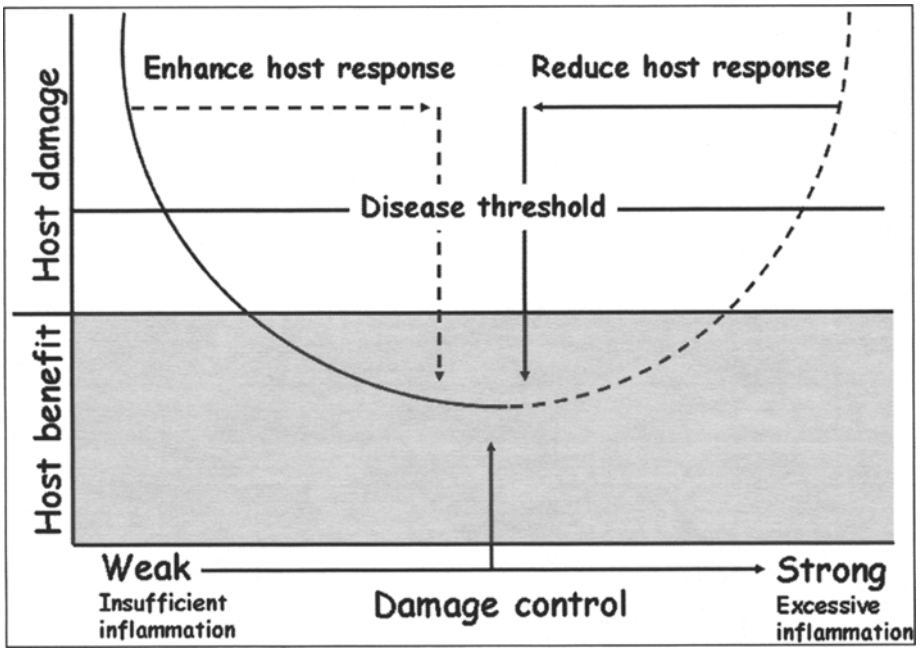

Figure 5. Use of the damage-response curve to develop approaches to therapy and prevention of infectious diseases. Host damage can occur in the setting of either weak or strong host responses. A rational approach to therapy for diseases that occur in the setting of a weak response is to enhance the host response, as shown by the dashed arrows leading to the dashed curve. A rational approach to therapy for diseases that occur in the setting of a strong response is to reduce the host response, as shown by the solid arrows leading to the solid curve.

individual microbes or complex interactions with the microbial community. Consequently, health is a condition whereby there is no disease. The state of no disease de facto includes microbes in both commensal and colonizing states, but the damage resulting from the host-microbe interactions is below the disease threshold. However, the same microbe-host interactions that are associated with health can lead to disease in situations of either a weak or excessive immune response. When an individual develops acquired immune deficiency, the same resident microbes with which they interacted in a state of normal immunity and health no longer subject to immune regulation or control and interactions with them can now cause disease. At the other end of the spectrum, an excessive immune response triggered by loss of immune regulation, or perhaps transient interaction with a microbe or allergen, could cause disease by damaging tissues in response to the presence of microbial antigens. Furthermore, immune responses to certain microbes can cause qualitative and quantitative changes in the immune response that predispose to allergic diseases. For example, experimental *C. neoformans* infection in rats does not cause clinical disease attributable to the fungus but elicits an immune response that predisposes to allergic airway disease.[25]

The simplicity and flexibility of the damage-response framework allows it to coexist easily with other views of immunity such as the 'danger'[26] and 'hygiene'[17] hypotheses. Although we note that the damage-response framework does not depend on these hypotheses for its ability to accommodate their views, its ability to incorporate them provides a measure of reassurance for its veracity. In this regard, we note that some types of host damage are analogous to the 'danger signals' postulated to elicit immune responses by Matzinger.[26] On the other hand, the damage-response framework view that health is found at the vertex of the parabola (U-curve) which corresponds to the nadir of host

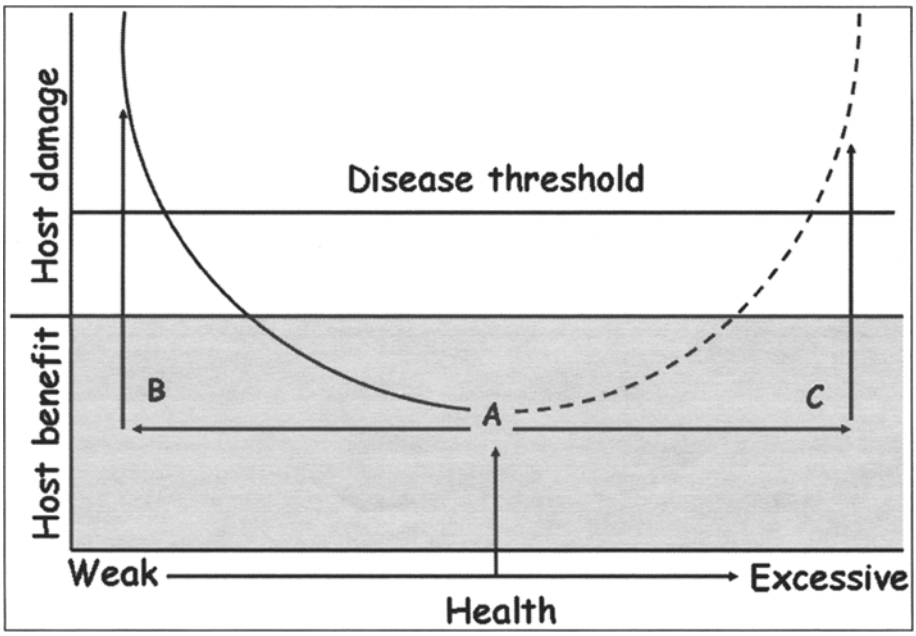

Figure 6. Use of the damage-response curve to illustrate hypothetical outcomes of human host-microbiota interactions. Host damage is portrayed as a function of the host response, whereby health is represented by an aggregate host response that controls microbiota-microbiota and microbiota-host interactions to provide a host benefit (A). Host damage occurs when the host response becomes more singular, either weak due to an insufficient response to the microbiota or the loss, disruption or dysregulation of host-microbiota or microbiota-microbiota relationships (B), or excessive due to a disproportionately strong response to the microbiota or the loss, disruption or dysregulation of host-microbiota or microbiota-microbiota relationships (C).

damage is echoed by the 'hygiene hypothesis' which posits that health requires longstanding and continued interactions with microbes to forestall the development of allergic and atopic diseases. Since the human host is in contact with thousands of microbes and for each host-microbe interaction there is an appropriate damage-response curve, one can easily imagine that the net aggregate of these responses gravitates towards a mean of minimum damage to the host. Hence, we posit that the aggregate curve of all the individual host-microbe interactions between an individual and its associated microbiota is a U-shaped curve with the condition of health requiring many types of immune responses which serve to control the microbes and to balance one another (Fig. 6).

References

1. Casadevall A, Pirofski L. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Immun 1999; 67:3703-13.
2. Casadevall A, Pirofski L. The damage-response framework of microbial pathogenesis. Nat Rev Microbiol 2003; 1:17-24.
3. Pirofski L, Casadevall A. The meaning of microbial exposure, infection, colonisation and disease in clinical practice. Lancet Infect Dis 2002; 2(10):628-35.
4. Rangel-Frausto MS, Wiblin T, Blumberg HM et al. National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to Candida species in seven surgical intensive care units and six neonatal intensive care units. Clin Infect Dis 1999; 29:253-8.
5. Blumberg HM, Jarvis WR, Soucie JM et al. Risk factors for candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. The National Epidemiology of Mycosis Survey. Clin Infect Dis 2001; 33(2):177-86.
6. Spellberg B, Powers JH, Brass EP et al. Trends in antimicrobial drug development: implications for the future. Clin Infect Dis 2004; 38(9):1279-86.
7. Armstrong D. History of opportunistic infection in the immunocompromised host. Clin Infect Dis 1993; 17(suppl):S318-S321.
8. Casadevall A, Pirofski LA. What is a pathogen? Ann Med 2002; 34(1):2-4.
9. Casadevall A, Pirofski L. Host-pathogen interactions: the attributes of virulence. J Infect Dis 2001; 184:337-45.
10. Casadevall A, Pirofski L. Host-pathogen interactions. II. The basic concepts of microbial commensalism, colonization, infection and disease. Infect Immun 2000; 68:6511-8.
11. Dethlefsen L, Eckburg PB, Bik EM et al. Assembly of the human intestinal microbiota. Trends Ecol Evol 2006.
12. Eckburg PB, Bik EM, Bernstein CN et al. Diversity of the human intestinal microbial flora. Science 2005; 308(5728):1635-8.
13. Palmer C, Bik EM, Eisen MB et al. Rapid quantitative profiling of complex microbial populations. Nucleic Acids Res 2006; 34(1):e5.
14. Mutch DM, Simmering R, Donnicola D et al. Impact of commensal microbiota on murine gastrointestinal tract gene ontologies. Physiol Genomics 2004; 19(1):22-31.
15. Mazmanian SK, Liu CH, Tzianabos AO et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122(1):107-18.
16. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? Trends Microbiol 2004; 12(12):562-8.
17. Bufford JD, Gern JE. The hygiene hypothesis revisited. Immunol Allergy Clin North Am 2005; 25(2):247-vi.
18. Kitagaki K, Businga TR, Racila D et al. Intestinal helminths protect in a murine model of asthma. J Immunol 2006; 177(3):1628-35.
19. Anlar FY, Kabasakal E, Karsi R. Tuberculosis and atopy: a study in an endemic area. Respir Med 2006; 100(9):1647-50.
20. Casadevall A, Pirofski LA. The weapon potential of a microbe. Trends Microbiol 2004; 12(6):259-63.
21. Casadevall A, Pirofski L. Fungi as biological weapons. Med Mycol 2006; In press.
22. Pirofski L, Casadevall A. Immunomodulators as an antimicrobial tool. Curr Opin Microbiol 2006; In press.
23. Committee on New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation. Treating infectious diseases in a microbial world: Report of two workshops on novel antimicrobial therapies. Washington, DC: National Academies Press, 2006.
24. Casadevall A, Pirofski L. A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. Advances Immunol 2006; In press.

25. Goldman DL, Davis J, Bommarito F et al. Enhanced allergic inflammation and airway responsiveness in rats with chronic Cryptococcus neoformans infection: potential role for fungal pulmonary infection in the pathogenesis of asthma. J Infect Dis 2006; 193(8):1178-86.
26. Anderson CC, Matzinger P. Danger: the view from the cliff. Semin Immunol 2000; 12:231-8.