OPINION/HYPOTHESIS

Thinking Globally, Acting Locally: Harnessing the Immune System to Deal with Recalcitrant Pathogens

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ABSTRACT **Traditional approaches to harnessing the immune system to confront infectious diseases depend on vaccines, which have generally proven highly effective, but for many infections these either are not available or are of limited effectiveness. Although antibiotic therapy has been extremely successful in reducing the burden of bacterial disease, the emergence of resistance among several important pathogens threatens to undermine this accomplishment, and despite some successes chemotherapeutic treatments for viral, fungal, and parasitic infections are more limited. Understanding the mechanisms whereby pathogens manipulate the immune system to favor their survival, or exploit weaknesses in host immunity, can lead to novel approaches for the treatment of infections by redirecting host immune responses against the pathogen. Such treatments may be most effectively applied at the mucosal locations which are frequently the sites of initial infection and may also suggest new approaches for vaccine development.**

For the past ~70 years, bacterial infections have been treated largely by means of antibiotics, generally with spectacular success, and as a result, at least in affluent countries, the threat of infectious disease has been dramatically diminished. However, as the World Health Organization, the U.S Centers for Disease Control and Prevention (CDC), and the U.S. National Institutes of Health are now warning, the emergence of multiple-antibiotic-resistant strains among several significant pathogens poses serious threats to human and animal health (1–3). While it is possible that entirely novel classes of antibiotics are waiting to be discovered on tropical forest floors or in the ocean depths (or elsewhere), the current outlook for new antibiotic development is bleak. The recent discovery of teixobactin (4) serves to underscore this view, for while it represents a novel class of antibiotic derived from an uncultivable soil microorganism, it is effective only against Gram-positive bacteria, and its clinical applicability is yet to be demonstrated. While initial findings suggest that the emergence of resistance may be unlikely, experience suggests that it would be imprudent to rely on this, at least until more evidence becomes available. Drugs that are effective against viruses and eukaryotic pathogens (fungi, protozoa, and helminths) are fewer and of variable efficacy, and they are also subject to the emergence of resistance traits, most notably in the case of malaria, where resistance to artemisinin appeared sooner than anticipated.

However, we do have within us an extraordinarily capable and adaptable system that has evolved to provide protection against infections—the immune system. This is amenable to exploitation to enhance protective immunity against a potentially unlimited variety of pathogenic agents. Traditionally, enhancing immunity has meant the use of vaccines, which are acknowledged to be one of the most successful of all medical inventions, although other modalities of immune intervention are now being explored, especially for the treatment of noninfectious conditions. It has become increasingly clear that pathogens protect themselves against their hosts' immune defenses by a variety of ingenious strategies. Antigenic variation has often evolved as an escape mechanism that enables the pathogen to evade specific adaptive immune responses, whether antibody or T cell mediated. Other, more broad-based strategies include countermeasures against immune defense mechanisms, such as resistance to phagocytic or complement-dependent killing, export pumps that eliminate various antimicrobial peptides, and enzymes that inactivate host effector molecules. Such immune evasion strategies can be considered "reactive" in the sense that they operate against the defenses that the host deploys against the pathogen. In addition, some well-adapted pathogens have evolved proactive mechanisms to avoid inducing or even to subvert for their own benefit the hosts' adaptive immune responses in the first place. Yet other pathogens succeed by exploiting inherent weaknesses in their hosts' defenses. Elucidation of these mechanisms of immune interference or exploitation by pathogens should afford novel opportunities to countermanipulate the immune system and thereby induce effective responses against the pathogens. Because such interventions rely on the ability of the adaptive immune system to mount a seemingly limitless array of specific defenses against pathogens, they are mechanistically distinct from the action of antibiotics, and therefore represent a different approach to the treatment of infectious disease.

Several examples of new immune-based interventions have come to light recently, collectively suggesting an alternative to focusing treatments directly on pathogens which have proven adept at evolving escape mechanisms to deal with such agents. As documented in a recent report from the CDC (2), several significant multiple-antibiotic-resistant bacterial pathogens are challenging the chemotherapeutic paradigm that we have been relying on, and even if new antibiotics are developed, experience suggests that these microbes will quickly evolve mechanisms that will soon render them ineffective. It is therefore time to think of and exploit new ways of dealing with the age-old problem of infectious disease. These become possible with increased understanding of how pathogens interact with their hosts in a two-way dynamic fashion.

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No longer is it sufficient to view microbial pathogenicity as a simple linear process in which the pathogen attacks the host and the host reacts and deploys defenses, which the pathogen then reacts to by putting up its own defenses and counterattacking, and so forth. Instead, it has become clear that both pathogen and host have sensory mechanisms that quickly detect the presence of each other and elicit signaling pathways leading to complex bidirectional responses. The pathogen attempts to elicit from the host responses that will favor its survival, including a supply of essential nutrients, while the host attempts to protect its tissues from harm, maintain homeostasis, and ultimately eliminate or neutralize the pathogen. The concept of the "damage-response framework" (5) is apposite, as the outcome may be an emergent property of the interaction between host and pathogen, not easily predictable from separate consideration of their characteristics.

A particular case in point is *Neisseria gonorrhoeae*. It has long been known that, unlike many well-known infectious diseases, gonococcal infection (gonorrhea) does not induce a state of protective immunity against repeat infection, which is relatively common. Conventional thinking suggests that the lack of protective immunity arises from the extraordinary capacity of *N. gonorrhoeae* for antigenic variation involving most of its major surface antigens, many of which can also be variably expressed through phase variation. For example, the major outer membrane porin protein is polymorphic, the opacity (Opa) proteins are encoded by genes at multiple loci which are subject to phase-variably controlled expression, the lipooligosaccharide glycan chains depend on the expression of glycosyltransferases that are phase-variably expressed, and pilus expression depends on recombination of gene segments at an expression locus (reviewed in reference 6). This has undoubtedly contributed to the failure of previous attempts to create a vaccine, most notably the gonococcal pilin vaccine, which induced protective antibodies against strains bearing the homologous pilus but was ineffective in clinical trial, as naturally occurring strains express unpredictable pilus variants (7).

However, recent findings reveal that, in addition, *N. gonorrhoeae* can proactively direct the pattern of host immune response in favor of its own survival but to the detriment of the host (8). Thus, *N. gonorrhoeae* selectively elicits Th17-driven innate defense mechanisms that it appears able to resist (at least partially) while concomitantly suppressing Th1- and Th2-driven adaptive immune responses that might be effective in eliminating it. The mechanisms by which *N. gonorrhoeae* accomplishes this manipulation of the host's immune response include the induction of the immunoregulatory cytokines transforming growth factor β (TGF-β) and interleukin 10 (IL-10) and possibly also type 1 regulatory T (Tr1) cells, which suppress adaptive immune responses governed by Th1 and Th2 cells (9, 10). We have found that these effects can be reversed by administering neutralizing antibodies to TGF-β and/or IL-10 or by the local application of IL-12 in a sustained-release formulation (11). Both of these types of treatment induce Th1-driven specific immune responses, including the generation of gonococcus-specific antibodies in serum and genital secretions, and the establishment of immune memory. These responses accelerate the clearance of *N. gonorrhoeae* in a murine model of female genital tract infection. Furthermore, reinfection with homologous or even heterologous strains several months later results in recall of specific immunity such that the challenge is resisted, with the generation of anamnestic Th1- and antibody-mediated responses. In addition to suggesting novel modes of therapeutic treatment of gonococcal infection with the added advantage of inducing prophylactic protection against reinfection, these findings inform novel approaches to gonococcal vaccine development. The need for such new treatments is underscored by the CDC's report that places *N. gonorrhoeae* among the top three pathogens meriting urgent action (2).

Several other organisms appear to exploit IL-10 as a strategy for enhancing their own survival. These include *Bordetella*, infection with which induces IL-10-driven suppression of immune responses (12, 13). Likewise, murine genital infection with *Chlamydia* elicits IL-10, which appears to suppress the development of Th1-driven protective immunity (14, 15). Although antibiotic treatment continues to be effective against *B. pertussis* and *C. trachomatis*, counteracting IL-10, whether by the local application of microencapsulated IL-10-neutralizing antibodies or IL-12 (11) or other means, might afford an alternative therapeutic strategy. Malaria infection is also associated with high levels of circulating IL-10, which serves to limit the resulting immunopathology (16). However, subjects with malaria are more susceptible to infection with nontyphoidal serotypes of *Salmonella enterica* as a consequence of the suppression of the mucosal inflammatory response, and in a mouse model of dual infection, blocking of IL-10 reduced the spread of the *Salmonella* (17). These examples, however, reveal the dualistic nature of IL-10 responses, which means that caution is needed in seeking to develop treatments based on counteracting IL-10. However, it should be emphasized that the treatment of genital gonococcal infection by the application of either IL-12 or neutralizing antibodies to IL-10 and/or TGF-β involves three key features: local application, low doses, and sustained-release formulation (11). These important considerations may avoid the adverse, even dangerous, effects that can arise from the systemic bolus injection of IL-12 and other cytokines or their corresponding blocking agents. However, the safety and acceptability of administering such preparations will need to be established. This is especially true in the female reproductive tract, where prolonged alterations of the cytokine profile may affect physiological functions, including pregnancy, as well as impacting the normal microbiota.

It is possible that many other instances of the proactive manipulation of immune responses by pathogenic microbes (8) will be found, involving similar or different mechanisms. For example, it is clear that *Mycobacterium tuberculosis*, which has been associated with humans for a long time, manipulates immune responses in multiple complex ways which have frustrated efforts to achieve effective vaccination against it (18). Elucidation of the mechanisms involved should lead to additional approaches to combating bacterial, viral, or eukaryotic pathogens that involve novel strategies of immune enhancement distinct from conventional vaccination. It is also possible that this will inform novel approaches to vaccine development aimed at eliciting the type of responses that favor the host rather than the pathogen and at delivering the responses to the relevant site of infection.

Furthermore, similar strategies of local immune intervention might be applicable to other infections, especially those of mucosal surfaces. Two other distinct examples of such an approach to harnessing immune responses in defense against infections have already been described. One utilized a sustained-release microencapsulated formulation of a chemokine (CCL22) to recruit regulatory T (Treg) cells to a site of bacterially induced chronic inflammation, i.e., the gingival tissue, in both murine and canine models

of experimental periodontitis (19). The treatment resulted in elevation of Treg-associated anti-inflammatory molecules, decreased proinflammatory cytokines, and reduced alveolar bone loss in mice. In the canine model, similar local application of microencapsulated CCL22 led to the amelioration of clinical inflammation as well as reduced periodontal bone loss. In this connection, it is now apparent that a major periodontal pathogen, *Porphyromonas gingivalis*, is able to manipulate the host's response, leading to a dysbiosis of the gingival microbiota that not only promotes its own survival but also results in the destructive inflammation of the periodontal tissues (20). Therapeutic measures aimed at controlling the inflammation and correcting the dysregulation of the immune response may therefore be an effective approach to treatment (21).

Another example is the topical application of the chemokines CXCL9 and CXCL10 to recruit effector T cells in the female genital tract after the administration of a vaccine against herpes simplex virus 2 (HSV-2), in a strategy described as "prime and pull" (22). Here, priming consisted of conventional parenteral vaccination, which induced anti-HSV $CD8^+$ T cells, but these were not recruited to the genital tract to provide protection against HSV challenge. Intravaginal administration of CXCL9 and CXCL10 provided the "pull" to recruit the effector Th1 and activated $CD8^+$ cells (which express the chemokine receptor CXCR3) to the required location for long-term protective immunity. In this example, the chemokines were applied in free soluble form. It is not known whether their application in a sustained-release formulation would be advantageous in facilitating uptake, as suggested by the finding that hydrophilic nanoparticles are readily taken up in the female genital tract epithelium (23). It is also possible that other immune effector agents could be delivered to induce the local production of required chemokines. This HSV model illustrates a common problem in immunization, especially in the context of local mucosal infections: induction of an appropriate response alone is insufficient; the effector cells or molecules must be delivered to the relevant site of infection to achieve protection.

The search for novel antibiotics must unquestionably continue, with renewed vigor and utilizing novel strategies such as have met with success in the discovery of teixobactin (4). Likewise, new and improved vaccines should be pursued by exploiting novel approaches, including "reverse vaccinology" and genome mining to discover new antigens, approaches that have proven successful in the development of a new vaccine against group B *Neisseria meningitidis* (24). However, there is also scope for the development of different approaches to the treatment of infections, aimed at redirecting host immune responses away from the counterproductive responses elicited by pathogens for their own benefit and into protective modes. These approaches will be complementary to the existing chemotherapeutic treatment of infections and may succeed when antibiotic treatments are inadequate and conventional vaccines are unavailable.

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