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Rostrum

Innate immune activation as a broad-spectrum biodefense strategy: Prospects and research challenges

Charles J. Hackett, PhD *Bethesda, Md*

Biodefense strategies require protection against a broad and largely unforeseen spectrum of pathogens—the forte of innate immune system defenses—that have evolved over millennia to function within moments of encountering either ancient or newly emerging pathogens. Although constitutive, the innate immune system is activated by the presence of microbes or their products, providing a rationale for a potential biodefense strategy. Both prophylactic and postexposure strategies involving innate immune stimulation have been shown to be plausible to prevent or ameliorate infections in animal models. Innate immune-activating compounds based on conserved microbial components recognized by toll-like molecules and other receptors could be synthesized and delivered like drugs by using an entirely different strategy from conventional vaccination. However, important theoretic and practical questions emerge about developing and deploying innate immune protective strategies for biodefense. This rostrum discusses prospects and problems in the overall approach itself. Important topics include microbe-specific issues about innate immune system effectiveness against highly virulent pathogens and general questions, such as whether innate immune responses will be safe and effective if used in a diverse human population of different age groups and with different genetic makeups.
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The powerful protective capability of innate immune defenses has not always been fully appreciated, likely because of its very success in shielding the body from an enormous array of potential infections. Backed by much new information on the molecular and cellular makeup of our inborn defenses, innate immunity is now seen as the major mechanism to prevent footholds by invading microbes, slowing and containing them until adaptive immune T and B cells can respond and clear the infection. Analyses of the human genome continue to identify new genes serving innate immune functions.[1] The fundamental role of innate immunity in host defense is well reflected by the presence in bacterial, viral, and parasitic pathogens of elaborate mechanisms for the evasion of innate immunity (reviews in *Nature Immunology* volume 3, November, 2002). Microarray analyses reveal numerous and highly significant changes in gene transcription in cells of the innate immune system after exposure to pathogens,[2] but at the same time, mutations in single genes of innate immune receptors might greatly increase the risk of infection or sepsis.[3,4]

Abbreviations used
NK: Natural killer
NOD: Nucleotide-binding oligomerization domain
TLR: Toll-like receptor

The needs of biodefense to protect against a broad and largely unforeseen spectrum of pathogens has provided impetus to develop rational methods to call into play innate immune defenses (discussed below).[5,6] Unlike the adaptive immune system, on which current vaccination and immunotherapeutic approaches are based, the innate immune system has evolved over millennia to function within moments of encountering either ancient or newly emerging pathogens. Although constitutively in place, the innate immune system is activated by the presence of microbes or certain of their products, providing a rationale for a potential biodefense strategy. Both prophylactic and postexposure strategies involving innate immune stimulation have been shown to be plausible to prevent or ameliorate infections in animal models.[7-12] Furthermore, many of the microbial compounds that activate innate immunity can be synthesized and optimized as artificial molecules. Examples include LPS, double-stranded RNA, DNA oligonucleotides containing unmethylated CpG motifs,[13-15] or even analogs of the essential amino acid isoleucine,[16] suggesting that compounds more akin to drugs than vaccines might constitute the next generation of immune system activators (Fig 1).

However, important theoretic and practical questions emerge about deploying innate immune protective strategies for biodefense. The purpose of this rostrum is to discuss prospects and problems in the overall approach itself. Important topics include microbe-specific issues about innate immune system effectiveness against highly

From the Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases, National Institutes of Health.
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Reprint requests: Charles J. Hackett, PhD, Chief, Molecular and Structural Immunology Section, Basic Immunology Branch, Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases, 6610 Rockledge Dr, Room 3009, Bethesda, MD, 20892-6601.
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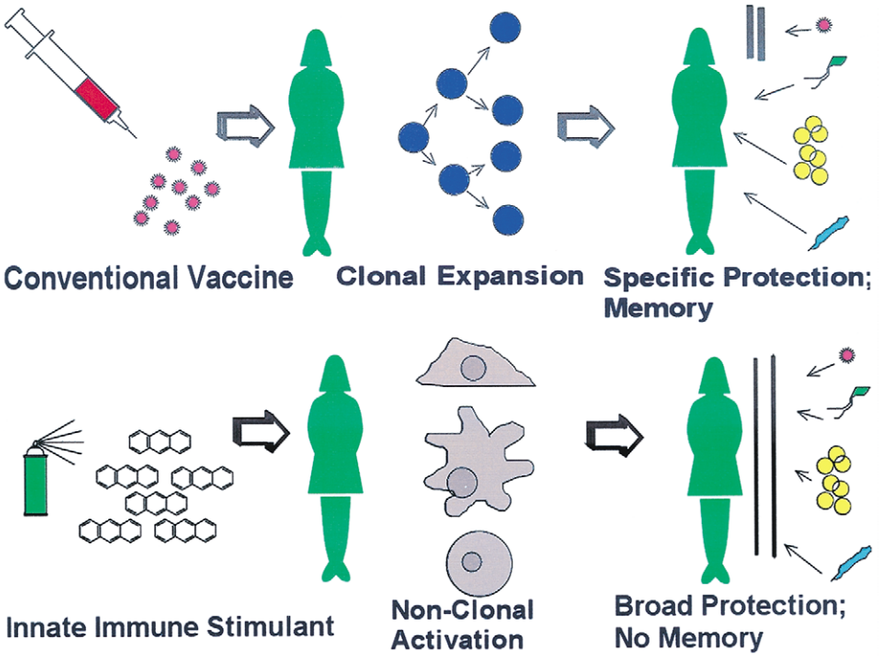

FIG 1. Comparison of proposed innate immune stimulation strategies with conventional vaccination. Vaccination uses whole pathogens or their subunits to expand antigen-specific B and T cells, leading to the development of specific and highly effective long-term protection over several weeks. Innate immune stimulants will likely be chemically synthesized compounds, possibly delivered by means of aerosol, that activate dendritic cells, macrophages, and other cells in a nonclonal manner aimed to provide rapid, broad, but impermanent protection against many potential pathogens.

virulent pathogens and general questions, such as whether innate immune responses will be safe and effective if used in a diverse human population of different age groups and with different genetic makeups.

ELEMENTS OF THE INNATE IMMUNE SYSTEM

Although the innate immune system functions in all tissues and organs, its role is especially prominent in those areas that are highly exposed to the external environment: the skin, digestive and genitourinary tracts, and airways. Not only are these regions of the body precisely those that are most vulnerable to attack by bioterrorist weapons, but they are also relatively accessible for potential interventions. Innate immune defenses are based on both the cells permanently located within tissues and on the migration of additional cells to the site of infection as needed. The innate immune system cellular network includes Langerhans cells of the skin, tissue dendritic cells and macrophages, and tissue-associated lymphocytes, such as natural killer (NK) cells and $\gamma\delta$ receptor T cells, which trigger these early responses to infection. Furthermore, many epithelial cells can sense and take action against microbes, including by means of secretion of antimicrobial peptides that directly act on the invaders, as well as upregulation of cytokines and chemokines that call additional cells into action, including signaling T and B cells that an infection is present. The key to rapid innate responses is the expression of highly specialized receptors, including the family of toll-like receptors (TLRs), which trigger cellular activation to molecules that constitute recurrent patterns of microbial structures. These targets include LPS, teichoic acid, flagellin, cell-wall lipoproteins, highly mannosylated polymers, and nucleic acids.[17] Furthermore, the immediate defensive steps triggered by innate immune activation also attack targets widespread among pathogens: antimicrobial peptide formation of pores in bacterial cell walls, IFN-α and IFN-β action against viral replication, and activation of the alternate complement pathway.[18]

Innate immune activation also triggers and paves the way for an adaptive immune response by antigen-specific T and B lymphocytes. Immature dendritic cells activated through their TLRs undergo a maturation process that includes migration to the local lymph node, expression of costimulatory molecules needed for lymphocyte activation, and processing and presentation of engulfed antigens (ie, protein antigens in the context of MHC class I and class II molecules and lipid materials by CD1 proteins). Thus innate immunity sounds the alarm that infection has occurred, summons defenses, and initiates antigen-specific T- and B-cell responses. For many years,

adjuvants have been used to enhance vaccination responses by a process now beginning to be understood as the innate immune triggering of adaptive immunity. The question is whether the early, broad, and more generic responses of the innate immune system can be rationally harnessed to protect before or after exposure to a variety of potential bioterrorist agents.

EVIDENCE THAT INNATE IMMUNE STIMULATION MIGHT PROVIDE PROPHYLACTIC PROTECTION, POSTEXPOSURE PROTECTION, OR BOTH

There are, at recent, clear examples of compounds targeted to specific innate immune receptors that provide rapid-acting protection against infection in animal models. Delivery of CpG nucleotides have been shown in mice to reduce the severity and time course of infection with the bacteria *Listeria* species, *Francisella* species, and *Mycobacterium tuberculosis*, as well as the parasites of malaria infection and *Leishmania* species.[7-12] The recent finding of immune protective effects in primates administered CpG before and shortly after infection suggests that human subjects might also benefit.[19] Importantly, the same study showed that macaques infected with simian immunodeficiency virus also exhibited reduced parasite lesions, supporting the notion that stimulation of innate immunity can have protective effects despite an infection that affects adaptive immunity. Protective effects correlated in some cases with the release of IFN-γ and IL-12,[12] but overall, the precise mechanisms of protection are not well understood.

It seems likely that other innate immune receptors in addition to TLR9/CpG should also be able to serve as targets for innate immune therapy. For example, synthetic immunostimulants that signal through TLR4 induced protective responses in mice against the influenza virus and *Listeria* species.[13] In a natural infection many distinct innate immune receptors contribute together to the innate immune response.[20-22] Current data indicate that stimulation of multiple TLR receptors leads to synergistic cytokine production.[23] This variety of signaling might lead to a highly effective protection that would be beneficial to mimic with immunotherapeutic approaches; one method might be to test the combination of differently targeted agonists, such as using TLR4 and TLR9 ligands together to more closely mimic the innate immune responses observed in natural infections. Therefore innate immune stimulation strategies have the potential to tap into complex and effective responses by using rationally designed molecules. Whether innate immune stimulation strategies would be effective depends on a number of factors: How do microbes activate innate immunity, and can that be effectively mimicked? Will innate immune defenses elicited by pure compounds elicit the full range of innate immune defenses? Will pathogen immune system evasion strategies negate attempts to use this approach? Will heterogeneity in health, age, and genes for innate immune system function in the human population prevent the use of innate immune system stimulation approaches?

INNATE IMMUNE RESPONSES TO BACTERIA

Bacterial triggering of innate immunity

The broad recognition of bacteria by the innate immune system reflects the reactions of an array of receptors specific for essential components of many bacterial cells. Bacterial cell-wall molecules interact with innate receptors found on human cells that include peptidoglycan recognition proteins for peptidoglycan recognition,[24] TLR2 for lipoteichoic acid and bacterial lipoproteins, TLR5 for bacterial flagellin, TLR9 for bacterial DNA, and CD14, LPB, and TLR4 for LPS.[17] Mannose-binding protein and lung surfactants react with bacterial outer capsules, whereas certain receptors found intracellularly react with phagocytosed bacteria, such as nucleotide-binding oligomerization domain (NOD) 1 and NOD2 in LPS recognition.[25] The relevant point is that many innate receptors participate together in the recognition of bacterial cells, and it is the integration of signals from many inputs, as well as their localization, that underlies innate immune effectiveness. Whether one or a few synthesized bacterial components could achieve innate immune activation to poise the body for effective responses emerges as a key area for study.

Innate immune defenses against bacteria

Antimicrobial compounds, produced both constitutively and in response to specific microbial activation, function both extracellularly and within phagosomes to destroy bacteria. Antimicrobial peptides can be present in high concentrations locally and destroy many bacteria by mechanisms distinct from those of antibiotics in current use.[26] Reactive oxygen and nitrogen radicals constitute major lethal defenses against intracellular bacteria. Bacteria coated with innate immune surfactants or mannose-binding protein, often including complement activated by the alternate (innate immune) pathway, are opsonized and more readily phagocytosed. It seems reasonable that at least the extracellular mediators of the innate immune system, such as innate opsonins, complement components, and certain antimicrobial peptides, could be effective antibacterial agents. For example, antimicrobial peptides have shown positive clinical results and can be synthetically modified to reduce toxicity to human cells while maintaining antibacterial activity.[27] With over 700 antimicrobial peptides from various species listed in a central database (http://www.bbcm.univ.trieste.it/~tossi/pag1.html), development of certain peptides into broad-spectrum treatments that would be at least partially effective against unknown or poorly defined bioterrorist agents seems possible and highly attractive. The question of selection of bacteria resistant to antimicrobial peptides needs to be taken into account. Multiple mechanisms of resistance to microbial peptides are known, including constitutive insensitivity and inducible modifications of the bacterial cell (reviewed in Yeaman and Yount[28]). For example, a virulence factor of *Staphylococcus aureus*, the action of which is to modify membrane lipids with L-lysine, was shown to contribute to resistance to antimicrobial peptide killing.[29] Biodefense strategies on the basis of only a few compounds, unlike the

natural situation that entails an array of local and systemic responses, raises the question of whether widespread use of such drugs might select resistant variants.

Bacterial innate immune evasion mechanisms

Some bacterial components tend to be more pathogen specific than LPS or flagellin, including outer membrane proteins, pili, and many virulence factors that represent relatively recent adaptations to pathogenicity and that might function in the evasion of innate immunity.[30] Enteropathogenic *Yersinia* species infection in mice provides a novel example of direct exploitation of innate immune receptors in immune evasion.[31] The *Yersinia* species virulence factor LcrV functions as a nonlipid-associated peptide agonist for the innate immune receptors CD14 and TLR2, resulting in the release of IL-10, an immunosuppressive peptide that increases the host susceptibility. The outer membrane protein Omp25 of *Brucella suis* inhibits TNF-α, a major innate and adaptive immune cytokine that combats *B suis* infection.[32] *Bordetella pertussis* possesses at least 6 known virulence factors the major effects of which are on macrophages, monocytes, and neutrophils, including the inhibition of cytokine release, oxidative responses, intracellular killing, chemotaxis, and phagocytic function.[33] Thus pathogenic bacteria possess multiple and highly effective means of counteracting innate immune activation and defensive functions. Indeed, certain bacteria might even take advantage of the local inflammation and cellular activation induced by innate responses to further their own survival and spread. For example, innate immune induction of TNF expression in human alveolar macrophages stimulates the intracellular replication of virulent *Mycobacterium tuberculosis*, even though overall the cytokine appears to have a protective effect.[34]

Therefore although the innate immune system possesses a wide array of microbial detection and host defense mechanisms, pathogen evasion is the norm. Protection by means of innate immune stimulation is likely to be of varying degrees of effectiveness depending on the specific agent, and highly virulent bacteria might well be those with the best innate immune evasion mechanisms. Stimulation of a specific innate immune countermeasure as a biodefense strategy needs to take into account whether it might be preferable to aim for a pathway other than that targeted by a particular microbe's evasion mechanism or whether that pathway might be precisely the one to overactivate because it is undeniably implicated in the pathogen's survival.

The 2001 anthrax attacks in the United States highlight the need for a better understanding of innate immune responses as a component of biodefense.[35] During inhalational anthrax, *Bacillus anthracis* spores undergo phagocytosis by alveolar macrophages, a key component of innate immunity in the lungs. Rather than being destroyed by those macrophages, however, the spores germinate within phagosomes and ultimately spread to the bloodstream.[36,37] Therefore as with some other microbial infections, a critical component of *B anthracis* pathogenesis is likely the active suppression of innate immune mechanisms.

B anthracis ultimately kills host macrophages to evade immune destruction.[38] Recent evidence suggests that the bacterial protein termed lethal factor selectively induces apoptosis of activated macrophages through proteolysis of key mitogen-activated protein kinase kinases and the resultant modification of internal signaling cascades.[39,40] Not only does this approach destroy the macrophages, but it prevents the release of key cytokines and chemokines that play key roles in stimulating other components of innate and specific immunity to anthrax. Earlier studies also showed that anthrax edema toxin could modify cytokine production through the toxin's adenylyl cyclase activity.[41]

The multiple and specific mechanisms used by *B anthracis* to avoid destruction by macrophages and to potentially modify cytokine responses to infection therefore appear to be key components of pathogenesis. Furthermore, those mechanisms stress the need to avoid or downregulate innate immune responses and suggest that interventions designed to improve innate immunity to infection could have therapeutic benefit.

INNATE IMMUNE RESPONSES TO VIRUSES

Molecular basis of antiviral recognition and defense

IFN-α, IFN-β, and, potentially, the newly described IFN-λ[42,43] are the major initial weaponry against most viruses. These cytokines are released from cells in response to innate immune signaling of the presence of viruses (reviewed in Samuel[44]). Viruses differ from bacteria in lacking the complex prokaryotic cell wall and membrane that bear unmistakable structural features that the innate immune system has evolved to recognize over millennia. Viruses have fewer common hallmarks for the innate immune system to latch onto; many have been introduced to human subjects only recently and might incorporate host glycosylation or proteins in their particles.

Viral double-stranded RNA, a molecular hallmark of infection present in intracellular stages of many human viruses, can be viewed as the viral counterpart of bacterial LPS or peptidoglycan as the major danger signal in many viral infections. TLR3 recognizes double-stranded RNA and its mimic compounds, such as poly inosine:cytosine, to trigger antiviral innate immune responses.[14] Because viral double-stranded RNA becomes available after virus entry and initiation of intracellular infectious stages, there might well be a time lag between exposure and cell infection before TLR3 triggering occurs.

Circulating molecules of the innate immune system might be able to act before TLR3 triggering by attaching to the external molecules of many viruses. For example, serum mannose-binding proteins bind high-mannose carbohydrates found on influenza and other viruses and mediate complement-dependent lysis of infected cells,[45] a function also carried out by lung surfactants A and D.[46] Furthermore, natural (pre-existing) IgM antibodies

secreted by CD5+ B-1–type B cells recognize repeating structures often found on microbes and participate in virus inactivation and triggering of adaptive antibody responses to viruses.[47]

Other TLRs have also evolved the ability to interact with specific viral components. TLR4 interacts with the F protein of respiratory syncytial virus.[48] TLR7 responds to the antiviral imidazoquinolones,[49] which potentially mimic as-yet-unidentified viral structures.

NK cells participate actively in specific antiviral detection and defenses. Murine cytomegalovirus specifically binds and activates mouse NK cells through the Ly49H receptor.[50] Human NK cells might also be triggered by viral hemagglutinins binding the sialic acid–bearing NKp44 and NKp46 receptors.[51,52] Furthermore, some viruses reduce the expression of MHC molecules on infected cells, an effective mechanism to evade adaptive immunity, but that can lead to lysis of the virus-infected cell by means of removal of the MHC molecules targeted by NK inhibitory receptors.[53] By virtue of their ability to recognize and destroy abnormal cells, NK cells might play decisive roles in innate immune defenses against many viruses.

These studies suggest that innate immune receptors for viruses can be identified and potentially targeted with compounds to induce protection. Recent studies show that poly inosine:cytosine and poly adenine:uracil treatment of mice through the respiratory tract 1 day before and 1 day after influenza infection reduced pulmonary virus titers compared with those of control animals 5 days after infection.[54] Presumably, additional triggering receptors and pathways for innate immune responses to viruses will be discovered that might serve as prophylactic and therapeutic stimulants for different viral infections.

Viral evasion of innate immunity

An impressive array of mechanisms by which viruses thwart innate immune recognition and destruction continues to be uncovered. Evasion of the effects of IFN-α and IFN-β is highly developed in many viruses and, in some smaller viruses, might be linked mainly to the function of single or a few viral genes. Genes functioning in IFN evasion include the NS1 protein of influenza A and B viruses; gp35 of Ebola; NSs of Rift Valley fever virus; the V, W, and C proteins of Nipah virus; and Newcastle disease virus V protein.[55-58] The poxvirus vaccinia has at least 4 gene products that counteract IFN-α and IFN-β, including a soluble receptor that soaks up the cytokine, a protein that inhibits IFN induction, and 2 gene products that affect the downstream pathway.[59] In addition, vaccinia also have the capability to thwart the effects on IFN-γ produced by cells, including NK cells, in innate immune responses.[60]

Recently, the retrovirus mouse mammary tumor virus was reported to make use of stimulation through TLR molecules to evade the immune system. Mouse mammary tumor virus persistence in certain mouse strains depends on production of the immunosuppressive cytokine IL-10 resulting from TLR4 stimulation,[61] which is reminiscent of the innate immune activation of IL-10 secretion by the *Yersinia* LcrV to further its infectivity by suppressing host immunity.[31]

Therapeutic approaches to activate innate immunity need to overcome viral evasion mechanisms. Questions about the potential effectiveness of the approaches include the following: can viral defenses be overcome if innate defenses, for example, IFN, are induced early in infection or in high amounts? If certain viruses typically induce a certain cytokine and yet have defenses against it, are different cytokines potentially more effective? Could the induction of strong antiviral responses predispose tissues for bacterial infections or autoimmune reactions?

POTENTIALLY HARMFUL EFFECTS OF INNATE IMMUNE RESPONSES

Not all innate immune responses are benign to the host. Potential consequences of triggering innate immune activation need to be considered. Major potential harmful effects are discussed below.

Septic shock

Over 1000 deaths annually in the United States result from sepsis,[3] a condition of runaway innate immune stimulation and response. Bacteria in the bloodstream lead to the release of large amounts of potent innate immune stimulators, including cytokines, chemokines, lipid mediators, and oxygen radicals.[3] Adenovirus viremia has also been reported to trigger a toxic shock–like syndrome.[62] Individual bacterial components, such as peptidoglycan, LPS, and CpG, model septic shock in animals.[63-65] Any strategy to activate innate immunity must address whether a septic shock–like condition might be in danger of being induced by administration in large amounts or while an individual is combating an infection.

Autoimmunity

Innate immune system activation by microbial products or adjuvants has been implicated in inflammatory processes that might be associated with pathologic responses of the adaptive immune system to self-proteins. T and B cells reactive to self-proteins are believed to be present in every individual but do not cause disease because they generally are not activated. T and B cells with autoimmune disease potential probably rarely encounter self-antigens presented in the context of costimulatory molecules, and their activity is controlled by peripheral immune tolerance, which includes active control by regulatory T cells.[66] However, in an autoimmune-prone mouse model of systemic lupus erythematosus, stimulation of B cells with receptors for self-immunoglobulin through TLR9 was sufficient to activate them to produce autoantibodies.[67] More generally, immature dendritic cells have the ability to take up materials from their environment but do not enter lymph nodes or express MHC class II or costimulatory molecules. But when signaled through innate immune recep-

tors, dendritic cells trigger a maturation process that results in a shift from antigen uptake to antigen presentation, with the dendritic cells migrating to lymph nodes and expressing costimulatory molecules and cytokines. A potential consequence of this enhanced level of stimulation is that self-antigens might be presented in the context of costimulatory molecules or a costimulatory cytokine environment. In this regard recent important studies have shown that normal immune regulation is susceptible to being overridden by innate immune stimulation. Activation of antigen-presenting cells by means of TLR stimulation stimulates IL-6 release, which temporarily inactivates $CD4^+$, $CD25^+$ T cells that ordinarily would dampen immune responses.[68] Although this process is thought to be part of the normal role of innate immunity that permits the establishment of effective adaptive immune responses, as would typically occur in vaccination or active infection, the overriding function of innate immunity might also contribute to innate immune-mediated damage or contribute to autoimmune disease activation. Certain individuals are prone to autoimmune diseases and whether they might experience increased risks from the use of innate immune activation requires analysis.

Activation of retroviruses

Signaling through TLR2 and TLR9 led to activation of HIV in a transgenic mouse model.[23] This observation needs to be followed up, especially because it might help explain the rapid reactivation of HIV in human subjects who have opportunistic infections that activate the innate immune system. Also, it raises the question about the use of strong innate immune activation as a biodefense strategy for HIV-infected individuals.

KEEPING INNATE IMMUNE RESPONSES IN CHECK

Multiple mechanisms prevent runaway activation of the innate immune system. Several negative signaling pathways are intimately tied to TLR signaling. The molecule suppressor of cytokine signaling 1 is induced by LPS and CpG treatment and acts on the intracellular signaling pathways of TLR4 and TLR9 to negatively regulate their activating functions.[69,70] The macrophage- and monocyte-expressed molecule IRAK-M prevents the dissociation of signaling kinases from TLRs, thereby effectively blocking TLR signaling.[71] Another protein, tollip, associates directly with TLR2 and TLR4 and inhibits signaling by blocking phosphorylation of other associated signaling molecules.[72,73] Additionally, an alternate and shortened form of the TLR signaling adaptor molecule MyD88 with inhibitory function arises after continuous innate immune stimulation, resulting in a transcriptionally controlled negative regulation of innate immune responses.[74] These molecules and additional mechanisms control responses to innate receptor activation and also contribute to the temporary tolerance of innate immune responses that appears to result from exposure to potent stimulants.[75] However, as demonstrated by Aderem et al, macrophages exhibit a sustained and continued activation during exposure to TLR agonists, although this is accompanied by a downmodulation of receptors[76] that makes the sustained response different from the initial response.[77]

Symbiosis with normal flora might also require the establishment of a form of innate immune tolerance. Continuous innate immune inflammatory stimulation must be avoided to coexist with normal microbial flora in the gastrointestinal tract. This accommodation might reflect at least in part that human intestinal epithelial cells are generally nonresponsive to bacterial ligands that stimulate TLR2.[7] Nevertheless, it is clear that innate immune responses to bacterial components in intestinal tissues are required for normal function, as evidenced by the association of Crohn's disease with a loss of function mutation in the molecule NOD2.[78,79] The presence of T cells with highly similar $\gamma\delta$ receptors situated throughout the lining of the gut might serve a counterinflammatory function to contribute to the establishment and maintenance of normal flora.[80] Any approaches designed to induce heightened innate immunity in the gut or other mucosal tissues will need to take into account the tolerance state involving normal flora and analyze the effect of the proposed strategy. Moreover, innate immune stimulation induced by treatment strategies must remain under the body's regulatory controls to avoid potential runaway responses that could lead to systemic shock. Potentially, along with the development of innate immune stimulants, there should also be measures available to counter innate inflammatory responses. It might be possible to develop potential controllers on the basis of innate immune receptors that are known. For example, inhibitory-suppressive motifs are known for DNA structures that can act dominantly over CpG stimulation, providing a potential antidote if immunostimulation by CpG sequences dangerously overresponded.[81,82]

HUMAN POPULATION DIFFERENCES IN INNATE IMMUNE GENES AND FUNCTIONS

Genetic mutations and polymorphisms in the human innate immune genes can underlie increased susceptibility to certain infections. For example, rare coding mutations in human TLR4 increased the risk for meningococcal sepsis.[83] Potentially, mutations in many innate immune receptors might have phenotypes related to disease. Mannose-binding lectin deficiency can be especially related to infections in childhood.[4] The importance for biodefense strategies is obvious: a potential strategy might not work or might yield unpredictable results in individuals with mutations or polymorphisms associated with the innate immune receptors targeted by a particular strategy. The number of affected individuals might be high in the aggregate. Certain polymorphisms in TLRs occur in several percent of the population.[84] Different ethnic groups might have distinct prevalence of altered forms of innate immune genes.[85] Given the large number of genes functioning in innate immunity, many individuals with mutations might be found throughout the human

TABLE I. Questions about innate immune stimulation for biodefense

1. Which innate immune receptors stimulate effective prophylactic responses to the broadest range of bacterial and viral pathogens?
2. How long does protection last?
3. Could innate immune therapy trigger harmful inflammation?
4. Will innate immune stimulation promote autoimmune reactions or retroviral activation?
5. Human genetic polymorphism and the innate immune system: How important a factor is it for innate immune therapy strategies?
6. Do the effects of age (ie, neonates through elderly) cause significant differences in innate immunity?
7. Can strategies be used with immunosuppressed individuals?

population, and identifying these mutations is an expanding area of research.

Redundancy in genes controlling broad innate immune function might circumvent problems from polymorphisms. Children who had defective signaling in the molecule IRAK-4 had a greatly increased risk for pyogenic infections but lacked the global disablement of innate immune responses perhaps expected of a mutation in a molecule central to innate immune signaling.[86]

The effect of age on innate immune function requires consideration in the broad application of innate immune approaches. At least some innate immune defenses are deficient in infants. Children generally achieve adult levels of soluble CD14 at about 4 months of age.[87] Probably for that reason, human breast milk contains high levels of soluble CD14, a receptor that functions with TLR4 in LPS responses, suggesting that passive bolstering of innate immune components are part of newborn protective mechanisms.[88] Elderly individuals might have some, but not all, innate immune functions exhibiting impaired activity.[89] Because innate immune stimulation is thought of as a broadly applicable strategy for biodefense and potentially a boon for vulnerable special populations, such as the old, the very young, and patients whose adaptive immune systems are impaired by chemotherapy or HIV, innate immune function in different age groups and disease conditions requires basic research studies.

CONCLUSION: RESEARCH QUESTIONS FOR INNATE IMMUNE ACTIVATION IN BIODEFENSE

The exciting possibility that powerful inborn defenses against infection might be manipulated to provide defenses against broad classes of bioterror agents and newly emerging infectious diseases, such as the severe acute respiratory syndrome coronavirus, is engaging researchers on many fronts of immunology and microbiology. Rational development of therapeutics on the basis of immune principles and pathogen susceptibility is ever more realistic because of a growing understanding of the pathogen genomes and virulence mechanisms, along with an explosion of information on innate immunity. Both basic biology and practical applications of the concept will benefit from the stimulus of biodefense research. Fundamental research that addresses questions about applicability, safety, and efficacy (Table I) lays important groundwork for innate immune stimulation as a counterweapon to bioterrorism.

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