The Innate Defense

Researchers hope that this "other" immune system will provide a target for "cutting-edge" therapeutics.

BY JEFFREY BACHA, OREOLA DONINI, KEN LEE, AND MONISHA SCOTT

When most people think about the human body being invaded by a foreign organism or a tissue gone awry, they think about the body's immune system recognizing a component of the invading or traitorous cell and launching a counteroffensive of white cells and antibodies. But in fact, they are only considering half of the story.

This scenario describes adaptive immunity, which relies on antigen-dependent immunologic memory and can take several days or weeks to develop. The other half of the immunological coin is the innate immune response, which keeps random microbes from becoming infectious agents. Elements of innate immunity are marshaled immediately in response to infection. Thus, by understanding its mechanisms, it should be possible to identify new medicines that selectively upregulate the infection-clearing aspects of the innate immune response while limiting harmful inflammation.

The double-edged sword

The innate immune response is a complex interactive network of cellular and molecular systems that provide the first line of host defense. The innate response recognizes and eradicates pathogens and harmful foreign molecules. Innate immunity is highly conserved and is present in even the simplest animals, including many that lack an adaptive immune system.

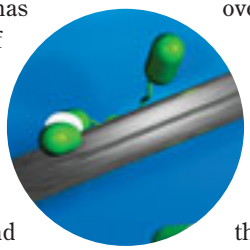
An innate immune response can be triggered by binding pathogen-associated molecular patterns or signaling molecules to pattern-recognition receptors such as Toll-like receptors (TLRs), mannose receptors, or complement receptors on the surface of host cells (*1*). These signaling molecules are present exclusively on microbes, including bacteria, parasites, viruses, and fungi. For example, lipopolysaccharide (LPS) present on Gram-negative bacteria, lipoteichoic acid on Gram-positive bacteria, and mannans on yeast cell walls all trigger a response from the innate immune system. The binding of signaling molecules to pattern-recognition receptors results in rapid marshaling of neutrophils, monocytes, macrophages, complement factors, cytokines, antimicrobial peptides, and acute-phase proteins in a complex and highly regulated response against the infection.

During an innate immune response, excessive production of certain inflammatory mediators and pro-inflammatory cytokines can lead to a cascade that, if left unchecked, causes tissue damage or sepsis, a potentially lethal condition. An uncontrolled inflammatory response can be triggered by bacterial components released during infection or by the bacteria themselves. Antibiotics can also stimulate the release of bacterial components via bacterial killing and therefore contribute to the risk of damaging

ILLUSTRATION: SCOTT NEITZKE

inflammation or sepsis (*2, 3*). Furthermore, patients cured of severe bacterial infections are often at a heightened risk of sepsis even after the infection has been cleared due to continued stimulation of inflammation by components of the infectious organisms.

Recent research into the mechanisms of innate immunity demonstrates the potential to selectively upregulate the infection-clearing aspects of the innate immune response while limiting harmful inflammation. The human peptide LL-37 has been demonstrated to upregulate the expression of chemokines in macrophages and in mice without stimulating the pro-inflammatory cytokine TNF-α (*4*). These results show that it may be possible to identify therapeutic compounds that stimulate innate immunity to recruit immune cells to combat infection while limiting inflammation-related tissue damage and sepsis. Companies such as Inimex Pharmaceuticals (*www.inimexpharma.com*) are exploiting these discoveries to develop new pharmaceuticals (see box, "Solving the mystery"). These medicines could enable strategies to treat or prevent a broad range of diseases, including bacterial and fungal diseases, viral infections, inflammatory disorders, and cancer (Table 1, *5*).



Clinical implications

Infectious diseases are the leading cause of death worldwide, and antibiotics are the third-largest sector of the global pharmaceutical market. In North America, pneumonia, septicemia, and infections of the lower respiratory tract are among the 10 leading causes of death. Each year in the United States, *Streptococcus pneumoniae* accounts for more than 135,000 hospitalizations and over 8400 deaths.

The overall incidence of bacterial infection in the developed world is expected to grow moderately in the near future; however, the incidence of drug-resistant infections is expected to rise more significantly because of the increased prevalence of drug-resistant phenotypes. A recent study determined that more than 40% of *Pseudomonas* strains isolated in North American hospitals demonstrated a multidrug-resistant phenotype (*6*). More alarmingly, a 1999 report by New York City's Public Research Institute indicated that more than 25% of all enterococcal infections in New York's intensive care units were vancomycin-resistant (*7*). With drug resistance becoming a crucial issue for all antibiotic classes, there is a significant need for

Solving the mystery

The Functional Pathogenomics of Mucosal Immunity (FPMI) project is a multicenter initiative sponsored by Genome Canada (*www.genomecanada.ca*), and it represents the largest functional comparative genomics initiative undertaken to date for the study of innate immunity. Collaborators in the project include Inimex Pharmaceuticals, Inc., Pyxis Genomics, Inc. (*www.pyxisgenomics.com*), the University of British Columbia (*www.ubc.ca*), Simon Frasier University (*www.sfu.ca*), and the Vaccine & Infectious Disease Organization at the University of Saskatchewan (*www.vido.org*).

FPMI project researchers are studying the interactions between pathogen, infected cell, and host immune system at the genomic level to determine which events are induced by the pathogen directly and which represent a protective or destructive host response, such as endotoxemia. The FPMI project will conduct more than 10,000 microarray studies over the course of three years, examining pathogen genomics and the genomics of the host response to pathogens in multiple species. Project researchers also will study novel peptides that are known to selectively enhance the innate immune response while suppressing certain inflammatory pathways.

The study of the interplay between the pathogen and the host involves initiating the interaction (i.e., applying a pathogen) and perturbing the resulting cascades. This can be achieved by perturbing the system with exogenous drug compounds to study differential expression patterns.

FPMI researchers are studying proteomic as well as genomic responses by specifically designing studies that evaluate the pathogens, host responses, and immunomodulatory perturbations at the protein level. These evaluations include not only classical biochemistry using commensurate cell lines and time points to the genomic evaluations, but also high-throughput proteomic analyses. The cellular evaluations are gradually expanded in complexity from simple-cell-only peptidic responses, to cell-plus-pathogen peptidic responses, and finally to multiple cell-plus-pathogen peptidic responses, allowing the complexity of the in vivo situation to be "rebuilt" from the simplest components and enabling the contributions of each cellular species to be identified.

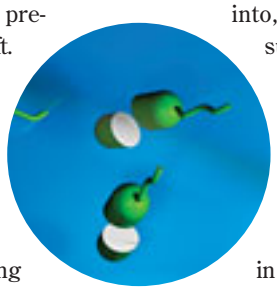
Although most systems biology research at this time is limited to cellular evaluations, the response of the whole organism and the correlation between the in vivo efficacy and genomic and proteomic responses will provide the ultimate value. FPMI researchers are also evaluating the pathogen, host, and peptidic responses in in vivo environments across multiple species.

To exploit the value of discoveries made in the course of the FPMI project, Inimex Pharmaceuticals has assembled a strong multidisciplinary capability in bioinformatics, structural biology, biochemistry, and computational chemistry. This interdisciplinary team forms the interface between the large amounts of data generated by the FPMI project and the company's drug development team. The team is structured cross-functionally to enable Inimex to accelerate the development of new therapies based on the selective upregulation of innate immunity.

In addition to participating in the FPMI project, Inimex has identified novel small peptides that selectively upregulate the infection-clearing aspects of innate immunity. These compounds have been studied at the genomic level as well as in cell-based and in vivo models. Inimex is advancing its initial product as a new therapy against nosocomial pneumonia.

novel approaches that avoid or overcome the mechanisms of microbial resistance.

New medicines that enhance innate immunity to prevent or treat infection may provide such a paradigm shift. Because such medicines would act on the host rather than directly against the microorganism, they would be less likely to engender the normal mechanisms of microbial resistance. Pathogen signaling molecules, which trigger an innate immune response, have also been shown to be essential to bacterial survival. Therefore, they are highly conserved either during environmental adaptation or the evolution of antibiotic-resistant phenotypes. An intriguing application of new medicines that selectively and safely upregulate the innate immune response would be to deliver them in conjunction with standard-of-care antibiotic therapy. Such medicines would complement antibiotic activity by boosting the host response to infection, thereby enhancing the use of traditional antibiotics that target the bacteria directly. Innate immune-boosting therapies would also have the added benefit of acting against emerging resistant strains before they had much of an opportunity to proliferate significantly.



Innate immunity can also safeguard the host against viral infection in a process similar to bacterial infection. For example, TLRs are involved in recognizing viral dsRNA, produced as a result of viral replication. This interaction leads to the activation of signaling pathways and the production of innate effector mechanisms. In addition, the central innate effector, NF-κB, has been shown to be an essential antiviral response against several viruses that infect humans, including parainfluenza virus type 3 and respiratory syncytial virus (*8*). The virion component, glycoprotein B, can initiate cellular transcription in host cells, which is analogous to the ability of the bacterial component, LPS, to initiate host cell changes without the whole bacteria (*9*). Immunomodulators such as LL-37 have been shown to selectively modify bacterial or LPS-induced host cell responses. Selective augmentation of the innate immune response would provide a new paradigm in antiviral therapy.

Preventive medicines represent another application of selective enhancement of the innate immune response. Products with this profile could be administered to healthy people before an anticipated encounter with, or entry into, an environment where there is a high risk of exposure to multiple or ill-defined pathogens. Such a setting includes travel to developing countries or entry into a hospital for surgery. These medicines could also become standard components of emergency-readiness packs prepared in response to potential bioterrorism threats.

Innate immunity also has a well-characterized role in the surveillance and rejection of tumors (*10*). Many studies have demonstrated the conserved nature of host defense genes that have dual roles in suppressing tumor growth and eliminating viral and bacterial pathogens. However, cytokine-based immunotherapies that upregulate host immunity against cancers have resulted in significant toxicity and modest clinical response in trials to date.

New understandings of host immunity may enable the interface between innate immunity and tumor suppression to be applied to the treatment and prevention of cancer. The development of new medicines that selectively activate key cells in the immune response to destroy tumor cells while suppressing harmful inflammation would lead to new anticancer protocols that could stand alone or be combined with other methodologies

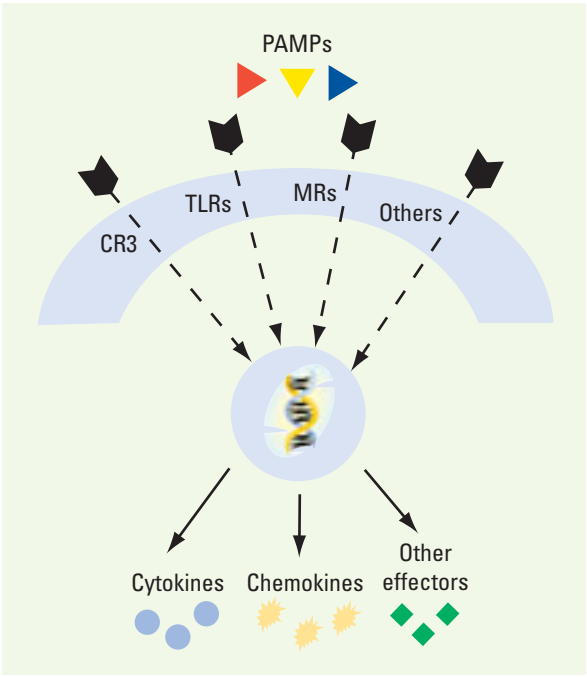


Figure 1. Innate immune response signaling. Components of invading pathogens (such as lipopolysaccharides, sugars, proteins, or nucleic acids, collectively termed pathogen-associated molecular patterns, or PAMPs) signal the initiation of the innate response. Multiple response cascades are activated upon the binding of PAMPs to pattern-recognition receptors (PRRs), receptors that are present in plasma in soluble form (e.g., mannan-binding lectin or LPS-binding protein) or found in the membranes of the cells that mediate the innate response, such as epithelial cells, macrophages, and neutrophils (e.g., type 3 complement receptor (CR3), Toll-like receptor (TLR), and mannose receptor (MR)). PRR activation launches a series of intracellular signaling events, ultimately leading to cellular activation and transcriptional changes that result in the production of the effector molecules of the innate response (including various chemokines, cytokines, and antimicrobial products).

Current approaches

Many "immunomodulatory" approaches have been pursued as potential therapies (Table 1). Immunomodulatory drug compounds can be loosely classed into three categories:

- low-molecular-weight plant compounds with "immune-enhancing activity";
- synthetic or purified macromolecular byproducts of bacterial lysis, such as lipopolysaccharides, oligonucleotides, and lipoproteins; and
- biologicals, such as cytokines and growth factors.

It is possible that several of the compounds in development as immunostimulators stimulate components of the innate immune response. For example, novel peptide compounds, CpG oligonucleotides, and synthetic derivatives of monophosphoryl lipid A have the ability to activate host immunity and are being developed

Table 1

Immunostimulatory drugs

Drug	Company	Status	Indication
IMP321	Immutep	Phase I	Antiviral
Leishmania elongation initiation factor (LeIF)	Corixa	Phase I	Antiviral, antibacterial
Hu14.18IL2	Lexigen Pharmaceuticals	Phase I	Anticancer
Abavca	Advanced Plant Pharmaceuticals	Phase I/II	Antiviral
AdIFNg	Transgene	Phase I/II	Anticancer
CpG 7909	Coley Pharma/Aventis	Phase I/II	Anticancer, antiviral
Beta-alethine	LifeTime Pharmaceuticals	Phase I/II	Anticancer
PEP005	Peplin Biotech/Allergan	Phase II	Anticancer
OK-432 (picibanil)	Chugai Pharma/Roche	Phase II	Macrocystic lymphangioma
Sho-siko-to	Honso Pharmaceutical Co	Phase II	Anticancer
HspE7	Stressgen/Roche	Phase II	Antiviral
ISS	Dynavax	Phase II	Asthma
Imreg	Imreg	Phase II	Antiviral
CpG-ODN	Tri-link Biotechnology	Phase II	Antibacterial
Romurtide	Daichi	Marketed	Antibacterial
RU 41740	Aventis	Marketed	Antibacterial
Pidotimod	Pharmacia	Marketed	Antibacterial
Monophosphoryl lipid A	GlaxoSmithKline	Marketed	Antibacterial
Bestatin (Ubenimex)	Microbial Chemistry Research Foundation	Marketed	Anticancer
Ampligen	Hemispherx	Marketed	Antiviral
Arbekacin	Meiji Seika	Marketed	Antibacterial

against a broad range of disease indications, ranging from bacterial infection to psoriasis. However, certain immunomodulators in development are known to be highly charged and may not be well tolerated in high or repeated therapeutic dosing. In addition, compounds acting through TLRs may upregulate cytokine production and increase the expression of potentially harmful inflammatory pathways associated with a broad innate response to infection.

Future directions

Controlled augmentation of host innate immunity is the basis of a new paradigm for treating and preventing a broad range of diseases. Because these medicines act on the host rather than directly against the pathogen, they avoid the normal mechanisms of drug resistance.

Studies using leading-edge genomic and proteomic techniques have begun to provide a new understanding of innate immunity systems biology. Research being conducted in leading academic and pharmaceutical company laboratories is unlocking mechanisms that will allow the treatment and prevention of disease by new medicines that selectively upregulate the infection-clearing aspects of the innate immune response. This research is providing the foundation for novel drug discovery platforms that will enhance the armamentarium of therapeutic options against inflammatory disease, cancer, and a broad range of infectious diseases.

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