

Are There Shared Pathways of Attack That Might Provide New Avenues of Prevention?

WORKING GROUP DESCRIPTION

Background

Microbial pathogens proceed through a series of general steps upon infecting hosts. A pathogen senses the host through surface receptors, which in turn trigger changes in the pathogen. These changes might include remodeling its surface and expressing proteins that adhere to the host or facilitate entry. Pathogens also deliver proteins and small molecules to their hosts that are sensed by host receptors and that alter basic host physiology, including signal transduction, the cytoskeleton, programmed cell death, and endocytic trafficking. Any one of these steps could be the target of a small molecule that would tip the balance against an infectious microbe.

The Problem

Can genomics help reveal the molecules involved in these steps of pathogenesis? Bacterial sensors can involve histidine kinases, cyclic AMP or cyclic di-GMP, proteins or toxins with enzymatic activity, and glycolipids and lipoproteins that trigger innate immune receptors. Comparative genomics of isolates with differing courses, of related pathogens with differing host range, or of commensals could help define the critical sensors and other genes critical for pathogenesis. Expression analysis of pathogens upon exposure to hosts could help define the critical modifications of the pathogen as it enters the host environment. Protein arrays, comparative proteomics using mass spectrometry, or two-hybrid systems might define ligands and protein interactions.

Differences in host response to pathogens due to host genetic variation might also reveal critical processes in pathogenesis. Resistance might come at any of many levels. Are there

populations with increased risk or resistance for classes of pathogens? These human variants could provide valuable insights into the infectious process. Analysis of critical host targets of microbial molecules in human or animal populations may provide evidence for natural selection of these targets and indicate alleles, which promote variability in the response to infectious diseases.

The group will consider these and other more novel approaches where genomics and host resistance may intersect across the full spectrum of microbial pathogenesis.

Initial References

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WORKING GROUP SUMMARY

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Summary

In November 2005 more than one hundred leaders from far-flung fields of science traveled to the National Academies Beckman Center in Irvine, California, to discuss genomics and its enormous implications for infectious disease. The interdisciplinary group hoped to determine whether microbial pathogens—a huge category that spans bacteria, viruses, and fungi—attack host organisms similarly. The answer? Yes.

Day 1: Host or pathogen, the genomic chicken or egg problem

Seated around a rectangular table in Southern California, the members of the working group fell into two camps: those most interested in the host and those expert in pathogens. The informal division echoed that of the greater scientific community, members of which have fanned out to research the common virulence factors of pathogens or the nature of the host response to infectious disease. Both parties have benefited tremendously from rapid whole-genome sequencing made possible by automation. Both are equally important to gaining a better understanding of pathogenesis. This last fact convinced the group to tackle the assignment from the angles of host and pathogen.

Despite the group struggling to find commonality among endlessly diverse pathogens, several concrete ideas began to form. Studying the nutritional needs of infectious agents, particularly their appetite for iron, was the first idea put forward. If most pathogens require this nutrient, would it be feasible to temporarily deprive the host of iron and kill the bugs, or at least “turn the rheostat toward the host”? Could chelation therapy, a controversial treatment that removes metals from the body, aid in this approach? Regardless of the answer, the group had successfully identified a common need among infectious agents.

The discussion soon migrated to viruses. This formidable class of pathogens is often inhibited by interferon, a key family of proteins that the human body uses to fight viruses on the cellular level and that the pharmaceutical industry synthesizes for antiviral therapies. Many viruses have adapted to evade interferon immune signaling or suppress its production. The catch is that different viruses employ different strategies. For example, one strain of the hepatitis C virus disarms interferon by simulating one of interferon’s molecular targets; therefore, despite

viruses sharing a similar mechanism, scientists couldn't simply target one common step of viral pathogenesis.

Tampering with the gene expression and replication functions of viruses posed another possibility. When viruses invade cells, they start replicating; therefore, halting this process would prevent the pathogen from gathering strength. Biotech companies have enjoyed some success stopping viral replication by developing compounds that block viral RNA.

Turning to the host for inspiration, the group considered pathogens' preferred mode of entry. The human body is home to several major microbial niches: the mouth, the intestinal tract, the skin, and in females, the vagina. These niches serve as common gateways to the host. The group thought skin cells are the most likely point of entry that can be fortified against microbial attack.

A second idea from the host vantage point was to monitor humans for clues in the form of Toll-like receptors and cytokines. Both types of proteins play a central role in the host's immune response. Toll-like receptors serve as the trigger, sensing the presence of microbes and signaling the immune system to take action. Cytokines follow close on their heels. These regulatory molecules are one of the first types of proteins the host releases after the immune system registers a threat.

Stanford scholar and researcher David Relman's conference tutorial on human microbial pathogens and commensals (or organisms that take part in a symbiotic relationship, in this instance, humans and the microbial flora found in their intestines) spurred the group's final idea. The relationship between host microbiota and infectious disease fascinated the team. Are people more susceptible to disease in the absence of natural microbiota? How do different circumstances alter the microbiota concentration in the host system? Could scientists identify the "good microbiota cops" to give to patients? All were good questions with no ready answers or clinical evidence.

Day 2: Science fiction, cytokines, and Saran Wrap

Two more ideas were discussed as the now familiar team reassembled. Taking a cue from science fiction, one group member wondered whether a cell flush with MHC-compatible inhibitors might be possible. MHC, or major histocompatibility complex, refers to the set of

human genes that code for antigens located on the surfaces of cells. His second idea found a more receptive audience. Building on the earlier discussion of cytokines, he proposed measuring these molecules in the blood and feces of the host, a sort of rapid detection system for infection. The trick was that the baseline level of these chemical messengers would differ from host to host, so healthcare providers would need this patient data on medical records before prescribing care. The other problem is that cytokines are ambiguous by nature; they indicate infection without identifying disease.

The group backed the cytokine idea with a small change. Instead of sampling blood and feces, why not take biopsies from infected patients? If biopsies were not an option, maybe good sources of secondary data existed, such as the National Institute of General Medical Sciences grant awarded to a group of scientists to study how the body reacts to injury on a molecular level.

Setting aside cytokines, the group members rehashed the concept of blocking potential points of entry. What they needed was the medical equivalent of Saran Wrap to preempt invasion. Even a semipermeable, short-term, synthetic barrier akin to Gore-Tex in theory would work. The only snag is that doctors might have a tough time persuading patients to wrap themselves in Gore-Tex or Saran Wrap.

With the clock ticking, the working group switched strategies. Rather than concentrate on the point of entry, the group decided to focus on the class of organisms it wanted to screen against. The team drafted the following outline of its kingdom approach for pathogens:

1. Conduct whole-genome-based comparisons by means of computer analysis for a class of organisms, such as fungi.
2. Identify those genes common to a class of organisms. For example, about 230 genes are present in fungi but not in humans or mice. In addition, many of these genes code for metabolic enzymes and exist as homologues, or similar genes in bacteria.
3. Filter out human genes to identify genes unique to organisms (in this example, fungi).
4. Analyze gene sets to determine whether any have been linked to virulence.
5. Knock out the selected gene in the pathogen and evaluate growth of mutant in vitro (culture) and in vivo (small animal).

6. Develop an in vitro assay for function of gene product.
7. Carry out high-throughput screening, utilizing the assay for gene product function.
8. Develop individual and cocktail drugs.

The next steps would be to modify this eight-step approach to include viruses and develop a viable plan from the host perspective.

Day 3: Finishing the task of innovative science

The final working group session opened with exciting news. Armed with a laptop and the extraordinarily broad, publicly available databases of genome sequencing projects, a group member uncovered some excellent protein targets for drug therapy the previous evening. These targets included HisG in bacteria, His1 in fungi, and others. Even better, the group thought that if it could find nonhuman proteins it could inhibit in fungi, it could apply that same technique to other classes of pathogens, including viruses.

To complete the assigned task, the group still had to return to the other party in host-pathogen interactions, namely, the host. This part was slightly hazier. Some group members did not see the utility of genomics to tailor therapy to individuals. They were more intrigued by the thought of treating patients with nonspecific immunotherapies, such as interferon, that prime the immune system in a general manner, or conversely, use pharmacological agents to interrupt these pathways (this approach would rely on genomics-type information to make diagnoses and institute appropriate treatment). The problem would be knowing when to dispense these therapies, or “when to apply the gas and when to apply the brake.”

The practical implication of this proposal was that healthcare providers would need a small collection of patient readouts that measured items indicative of infection at elevated levels, such as cytokines and signaling events from Toll-like receptor stimulation. If the patients’ readouts mirrored profile 1, for example, they would receive treatment X. If the readouts resembled profile 2 (or however many profiles were deemed necessary), they would receive treatment Y. The idea was that the universe of pathogens might be infinite, but the number of host responses and treatment options might not be. Richard Jenner and Richard Young’s 2005

Nature Reviews article, which catalogued 77 different host-pathogen interactions and identified a common host-transcriptional response, also provided some support for the group's view that hosts react similarly to a wide array of pathogens.

Discovering individual thresholds for and responses to infection constituted the most difficult task of the group's host strategy and would entail the collection of massive datasets involving many different populations. One group member suggested that they could collect these data by obtaining informed consent from emergency room patients and defining where these patients were in the course of a disease (retrospectively) and cross-correlate the genomics data with clinical care. Large datasets could be gathered from individual patients, provided that genomic-level observations could be made on single cells. To this end, the National Human Genome Research Institute awarded a grant to develop these technologies. Eventually these datasets could benefit doctors prescribing treatment in individual or pandemic settings.

Just as with pathogen-oriented strategies, the virtue of this approach is that by gathering genomic-level information, the group anticipated the identification of molecular pathways associated with diverse host cell functions, some of which could be modulated to favor quality outcomes for the infected host. Previously known, immunological pathways are one example of potential therapeutic targets. However, the group hypothesized that genomic-level information would provide additional therapeutic targets not necessarily related to immune responses, such as nutritional pathways or cytoskeletal elements. These would be defined as relevant through validation studies, and by definition, the relevance of these pathways to infectious diseases may have been unknown prior to gathering these kinds of datasets.

The other related but less developed host-centric idea was to prevent pathogen invasion by strengthening the host's natural mechanisms for preventing infection—barriers and clearance. Speeding up clearance of waste products and other materials through the gastrointestinal track would mean that pathogens would have less time to establish themselves. Only half-serious, the team thought that the clearance strategy might require more restrooms.

In their short time together, the members of the working group developed testable and workable strategies for blocking the pathways of attack shared by different pathogens. Although the kingdom approach for pathogens may prove more fruitful initially, optimizing the host response to infectious microbes through better therapies and other intervention could change patient care. The group acknowledged that its models would not address all diseases, patients, or

pathogens, but they were a start. Now it was time for the greater scientific community to pick up the ideas developed by this interdisciplinary team and build on them.