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Molecular Koch's Postulates Applied to Microbial Pathogenicity

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Microbial genetics and molecular cloning now permit us to routinely isolate specific genes from a variety of microbial pathogens. Obviously not all genes from pathogenic microorganisms play a role in pathogenicity or virulence. Just as Koch's postulates were formulated to identify the causal relationship between an organism and a specific disease, the notion is presented here that a form of molecular Koch's postulates is needed when examining the potential role of genes and their products in the pathogenesis of infection and disease.

Bacterial pathogens possess distinct genetic properties that provide them with a significantly greater capacity to compete with other bacteria, to gain a foothold within a specific host, to avoid normal host defense mechanisms, and, once established, to multiply. To the microbial geneticist, the challenge is to dissect these genes from the more common genetic traits found both in pathogens and in nonpathogens. This challenge is being met more and more frequently. Consequently, the classical approach of comparing pathogenic and nonpathogenic isolates of the same species has given way to precise mutational analysis utilizing insertional inactivation or site-directed mutagenesis. Molecular cloning permits us to isolate specific virulence genes, to focus on their function and structure, and to modify them in a precise way. Hence, one can now hope to routinely compare bacterial cell lines identical except for a single defined pathogenic property.

In some ways, our capacity to isolate genes has outstripped the experimental means to document the essentiality of a given genetic property to pathogenicity. Thus, the simple cloning of a gene considered important in pathogenicity, submitting it to functional analysis, and even sequencing it are not sufficient unless one can rigorously prove that the loss (or gain) of the gene in the species of origin has a well-defined effect. In my view it is imperative when pursuing the genetic analysis of bacterial pathogenesis to apply some molecular form of Koch's postulates.

I wish to thank the undergraduate, graduate, and postdoctoral students who have worked in my laboratory for the past two decades for their interminable questions that led in part to this paper.

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In our laboratory, we have defined these postulates as follows: (*1*) The phenotype or property under investigation should be associated with pathogenic members of a genus or pathogenic strains of a species. (*2*) Specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence. (*3*) Reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity.

Alternatively: (*2A*) The gene(s) associated with the supposed virulence trait should be isolated by molecular methods. Specific inactivation or deletion of the gene(s) should lead to loss of function in the clone. (*3A*) The replacement of the modified gene(s) for its allelic counterpart in the strain of origin should lead to loss of function and loss of pathogenicity or virulence. Restoration of pathogenicity should accompany the reintroduction of the wild-type gene(s).

These postulates place a heavy burden on an investigator. They insist that genetic manipulation of the microorganism is a prerequisite for success, and, of course, for some pathogens, such study is not yet possible. Moreover, for either alternative, it is essential that the test of pathogenicity be performed with the species of origin using a relevant model of pathogenicity. One must also take into account that it is possible to affect genes associated with pathogenicity indirectly; thus, precise characterization of structural genes and the information about genes affecting biosynthesis and regulation are mandatory to an adequate fulfillment of these postulates.

Because genetic manipulation is not yet available for all microorganisms and because there are some pathogens, e.g., the gonococcus, for which a relevant model of pathogenicity is not available, one could reasonably argue that the induction of spe-

cific antibody to a defined gene product that neutralizes pathogenicity or virulence should be an acceptable alternative to a molecular Koch's postulates. Such an approach has been and certainly is a reasonable alternative. To be sure, the evolution of vaccines against several common pathogens was based on such an immunologic approach to pathogenicity, and this approach is still being actively pursued. Of course, knowing how to protect against disease and understanding microbial pathogenicity are not necessarily equivalent. It is my bias that in the context of understanding microbial pathogenicity, the genetic approach is often the more precise and preferred method. I offer two examples to make my case.

First, it is a widely held view that bacterial pili are the means by which many microorganisms achieve their specific adherence to host cells in order to occupy a unique niche. Indeed, there are considerable data showing that in some cases immunization of animals with purified pili is protective against disease. The molecular analysis of bacterial adhesins has provided a more specific view. For the enteric bacteria, which cause urinary tract infection, the specific adhesin employed for binding to a specific host cell receptor is actually a gene product distinct from that specifying the pilus subunit. It is likely that the pilus and the specific adhesin are closely associated, and then often are co-purified. As the molecular analysis of the bacterial adhesins employed by enteric bacteria progresses, one begins to believe that plasmid-mediated pilin molecules may bind directly to their receptors while chromosomally mediated pilin molecules form a scaffolding for an adhesin encoded by a different structural gene. No matter, the available genetic and molecular data now paint a more precise picture of the pathogenic process and provide us with more specific targets for the achievement of protection against disease.

As another example, I offer some recent findings from my own laboratory regarding bacterial invasion. We recently reported the isolation of a single genetic locus, *inv*, from *Yersinia pseudotuberculosis*, which when transferred to *Escherichia coli* K12 permits this innocuous microorganism to effectively enter eukaryotic cells. We inactivated this gene by a well-defined mutation and exchanged it for the wild-type allele, to yield a *Y. pseudotuberculosis inv*$^-$ strain. However, this derivative still exhibits low-level entry and strong adhesion to the surface of cultured mammalian cells. In my laboratory, Virginia Miller subsequently identified a second genetic locus, *ail*, in all pathogenic *Yersinia* that mediates adherence to and entry into cultured epithelial cells. In addition Kathleen McDonough cloned a plasmid-mediated adhesin from the pathogenic members of the *Yersinia*. It is not clear that inactivation of any one of these determinants leads to a total loss of pathogenicity. An antiserum raised to the *inv* gene product does appear to be protective. Yet, how these genetic determinants contribute individually to pathogenicity and act in concert can be more readily achieved by genetic manipulation than by antibody neutralization. Moreover, it is important to see that we cannot claim to have fulfilled molecular Koch's postulates for the *inv* gene since it is arguable that a cell culture–invasion model is not a relevant substitute for invasion of whole animal tissue.

The Henle-Koch postulates were intended as a means of identifying the causal relationship between an organism and a specific disease. Koch recognized that the postulates were not rigid. The limiting factor even in Koch's time was the lack of suitable experimental animal models in which human disease could be reliably reproduced. Moreover, the inability to grow presumed pathogens, e.g., the leprosy bacillus, was as relevant in Koch's time as it is now. The evolution of thought regarding Koch's postulates is nicely documented by Evans [1]. Thus, the discovery of viruses and their role in disease underscored the limitations of the original postulates. It became clear, for example, that the establishment of a given virus with a disease syndrome was possible only under certain circumstances which included the immunologic status of the host. Similarly, Evans traces the establishment of "Koch-like" postulates to the immunologic proof of disease causation; to the search for the association of slow viral infection and chronic neurologic illness; and, more recently, the causal relationships between viruses and cancer as well as the causative factors in certain chronic diseases brought about by exposure to toxins. Clearly, the postulates in their original form or as modified for a given situation will only be as good as the available technology. By the same token, it must be possible to modify the postulates as new technology permits us to examine new aspects of the pathogen and the host.

I was asked to present an overview of the genetic and molecular means by which one may investigate microbial pathogenicity that could serve as a foundation for the discussion of the exciting develop-

ments taking place in the study of many bacterial pathogens. But it is no longer necessary to talk about the methods that might be applied to the study of bacterial pathogens; this symposium in itself is a model of how the newer methods of genetics, molecular biology, and immunology can be brought to the study of the basic biology and practical aspects of those microbes that contribute to human and animal disease. In fact, the study of pathogenicity at the genetic and molecular level has become increasingly refined over the past several years. To meet this growing sophistication, it is necessary to redefine a set of acceptable criteria that can be applied to the analysis of microbial pathogenesis. The molecular Koch's postulates described here were not intended to be anything more than an attempt to provide the basis of a dialogue among interested investigators. There is little doubt that some will find fault with the logic or wish to define their own criteria. For me these postulates underscore the need to define and refine genetic exchange mechanisms among microorganisms and for new and better animal models or, preferably, alternative models of microbial infection; I believe these are as much the keys to success in understanding microbial pathogenicity as is molecular cloning. The only point I wish to assert unequivocally is that it is no longer necessary to speak of the promise of molecular biology and genetics for the study of pathogenic microorganisms. That promise is already being fulfilled.

References

1. Evans AS. Causation and disease: the Henle-Koch postulates revisited. Yale J Biol Med 1976;**49**:175–95