PERSPECTIVES

Molecular Koch's postulates applied to bacterial pathogenicity — a personal recollection 15 years later

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Koch's postulates were derived from Robert Koch's work on infectious diseases, such as anthrax and tuberculosis, which still engage us to this day. These guidelines were an attempt to establish a standard for identifying the specific causation of an infectious disease and to convince sceptics that microorganisms could cause disease. They were also established to encourage an increasing number of novice microbiologists to use more rigorous criteria before claiming a causal relationship between a microorganism and a disease.

In 1988, I was asked to summarize a symposium on the then relatively new field of microbial pathogenesis and to comment on the contributions of molecular biology and genetics to the study of bacterial pathogenicity. In the summary, I expressed the view that it was possible to apply a kind of Koch's postulates to help characterize whether or not a particular microbial gene was an essential component of the ability of the microorganism to infect and cause disease in a particular host. When the proceedings of the symposium were published, I contributed a short paper entitled 'Molecular Koch's postulates applied to microbial pathogenicity'[1]. This general approach to experimentally define pathogenicity genes is often cited — sometimes favourably and sometimes critically (TABLE 1).

I have no desire to defend or to expand these postulates in any formal way. They served their function at the time as a working hypothesis for the study of the genetic and molecular basis of pathogenicity. As noted by Fredericks and Relman[2], "The power of Koch's postulates comes not from their rigid application, but from the spirit of scientific rigour that they foster. The proof of disease causation rests on the concordance of scientific evidence, and Koch's postulates serve as guidelines for collecting this evidence". Another interpreter of Koch, Alfred Evans (who modified the postulates to describe the use of immunological evidence for proof of disease causation[3]) noted, "…failure to fulfil the Henle–Koch postulates does not eliminate a putative microorganism from playing a causative role in a disease. It did not at the time of Koch's presentation in 1890, and it certainly does not today. Postulates of causation must change with the technology available to prove them and with our knowledge of the disease". It is in this spirit that Koch's postulates have been modified over the years to encompass viruses, obligate parasites, slow viruses (viruses that cause symptoms in an infected host long after the original infection and which progress slowly) and the microbial causation of cancer. More recently, the postulates have been invoked for sequence-based identification of bacterial pathogens, to resolve outbreaks of infectious disease and even to define the causation of certain non-infectious diseases. In discussing the molecular postulates, I have noted, "Clearly the (molecular) postulates in their original form, or modified for a given situation, will only be

as good as new technology permits us to examine new aspects of the pathogen and the host"[4]. It is this latter point I want to discuss here. There are new technologies that now allow us to monitor the host cell or the intact host to reveal the function of distinct bacterial virulence genes in a way that was not imagined in 1988. In the following sections, I use some examples mostly from my own laboratory to make this point, not because they are the best examples, but because I am most familiar with them.

The new era for gene identification

When the molecular Koch's postulates were formulated we were at the threshold of an explosion of new technology and an expansion of interest in microbial pathogenicity. There were relatively few journals that would publish, or indeed consider, papers about the biology of bacterial pathogens. This has all changed — now dozens of review articles are written on bacterial virulence each year, and papers devoted to the cell biology of host–pathogen interactions appear monthly. Furthermore, there are an increasing number of articles that discuss and debate the evolution of pathogenicity and how innate immunity is the evolutionary product of host–microorganism encounters[4,5,6,7].

Perhaps the most revolutionary change is the availability of the complete genomic sequence of at least one representative of the many disease-causing bacteria, viruses and large parasites[8]. Similarly, the complete genome sequences of several host species are now available, including humans, insect vectors, domestic plants and animals, and that all time favourite, the laboratory mouse. Pathogenicity islands have been discovered and, together with bacteriophage and plasmids, provide insights into horizontal gene transfer of bacterial traits that are often essential for microbial virulence[9,10]. Genomes do not necessarily reveal their secrets about pathogenic traits by simple visual inspection, or even by sophisticated bioinformatics analysis. The genomic information, combined with the use

Table 1 | **A comparison of Koch's original postulates and the proposed molecular Koch's postulates**

Koch's original three postulates	The proposed molecular postulates
The parasite occurs in every case of the disease in question, and under circumstances which can account for the pathological changes and clinical course of the disease.	The phenotype or property under investigation should be associated with pathogenic members of a genus or pathogenic strains of a species.
The parasite occurs in no other disease as a fortuitous and non-pathogenic parasite.	Specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence, or 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.
After being fully isolated from the body and repeatedly grown in pure culture, the parasite can induce the disease anew.	Reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity, or 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).

of innovative genetic and molecular tools — such as signature-tagged mutagenesis, *in vivo* expression technology, promoter traps using fluorescent reporters, differential subtractive hybridization, two-hybrid systems and, more recently, the use of DNA microarrays to survey differential expression on a global scale — has facilitated the discovery of potential pathogenicity genes (reviewed in REF. 11).

There is still the need for a type of molecular Koch's postulates to determine the importance of these genes in bacterial virulence, but the terrain is now more complex. In 1988, it seemed it would be possible to identify the genes that were most essential for pathogenicity on the basis of existing animal models of infection. However, like the increasing sophistication of genetic and molecular methods, we also became more sophisticated in models of infection and disease, and cell culture was increasingly used. There is considerable nervousness about just how relevant some of these models are for understanding infectious disease or the biology of pathogenicity — although the results from cell-culture infection models are often confirmed in disease models, this is not always the case.

The newer surrogate infection models, which use organisms such as nematodes and fruit flies, are also stimulating new research into understanding microbial pathogenicity[12,13]. Undoubtedly, there is, or will be, nervousness about the relevance of virulence genes that are identified as operating in the surrogate organism, but not in the host in which the disease is found normally. For example, a gene of the plague bacillus that is essential for the death of *Caenorhabditus elegans* does not fulfil molecular Koch's postulates when applied to a mouse model, but does when applied to *Yersinia pestis* infection of the flea vector[14]. I would still define this as an essential virulence factor; however, others might take a different, animal-disease-based view.

Clearly, many bacterial genes exist as a consequence of competition between microorganisms over billions of years, rather than having arisen 'specifically' to deal with host factors. These genes might subsequently (perhaps fortuitously) provide increased fitness to the microorganisms that possess them in a new environment, such as that found in a new host. Are these 'legitimate' virulence factors even if they satisfy the molecular Koch's postulates? In my opinion, yes. It can be argued that 'every gene' is an evolutionary artefact, and has been preserved because it conferred a competitive advantage in a particular environment that presumably, in many cases, preceded the first parasitic encounter with a host.

In animal-infection models, we have gone from the days of using LD_{50} measurements (the infectious dose that is lethal to 50% of the animals infected) as an estimate of the contribution of a particular gene to the infectious process, to using quantization of microbial tissue load in different organs over time. It has been recognized that death is too stringent an experimental end-point. Simply counting the number of dead carcasses does not always reveal the contribution of a particular gene to the overall virulence of a microorganism. A mixture of mutant and wild-type bacterial cells that are inoculated into a host helps to reduce the animal-to-animal variation, and to highlight circumstances where a mutation in a given gene might not totally reduce the capacity of a microorganism to cause infection or disease, but does reduce its relative fitness for survival in the host (reviewed in REF. 15). New technologies to identify potential virulence genes have caused us to confront the reality that although we might be able to define the relative essentiality of a given microbial gene for pathogenicity, the actual function of that gene might not be so obvious. Often, we cannot deduce the contribution of a given

bacterial gene to the infectious process simply by counting either the number of dead mice or the number of bacteria present in the infected organs. Furthermore, a particular gene might be used only during a defined period of the infectious process, which we still do not know how or when to measure. We need to find new methods to experimentally determine the role and function of the bacterial gene, and its time of expression during the infectious process.

Using the host to identify gene function

Transgenic and 'knockout' animals. If the contribution of the bacterial genes involved in virulence are complex, the genetic factors of the host are even more numerous, more complex and also strongly determine the outcome of infectious diseases that are caused by various pathogens. Yet, production of mouse mutants by gene targeting, positional cloning of host-resistance genes in mutant mice and mapping and characterization of quantitative-trait loci (QTL) that control the complex aspects of host–pathogen interactions have a tremendous potential to let us experimentally dissect the complex genetic system of host–pathogen interactions into single components (reviewed in REF. 16).

The genetic dissection of molecular pathways that are involved in the host defence against microbial pathogens has been extremely successful using gene-targeting approaches. One example of how the use of gene-knockout animals has provided us with new information about the function of specific bacterial virulence genes and their role in the infectious process is that mediated by *Salmonella enterica* serovar Typhimurium.

Salmonella pathogenicity island 1 (SPI1) encodes the components of a type-III-secretion apparatus, which allows the delivery of bacterial effector proteins to host cells[17]. The effector genes in SPI1 are primarily expressed during the initial gastrointestinal
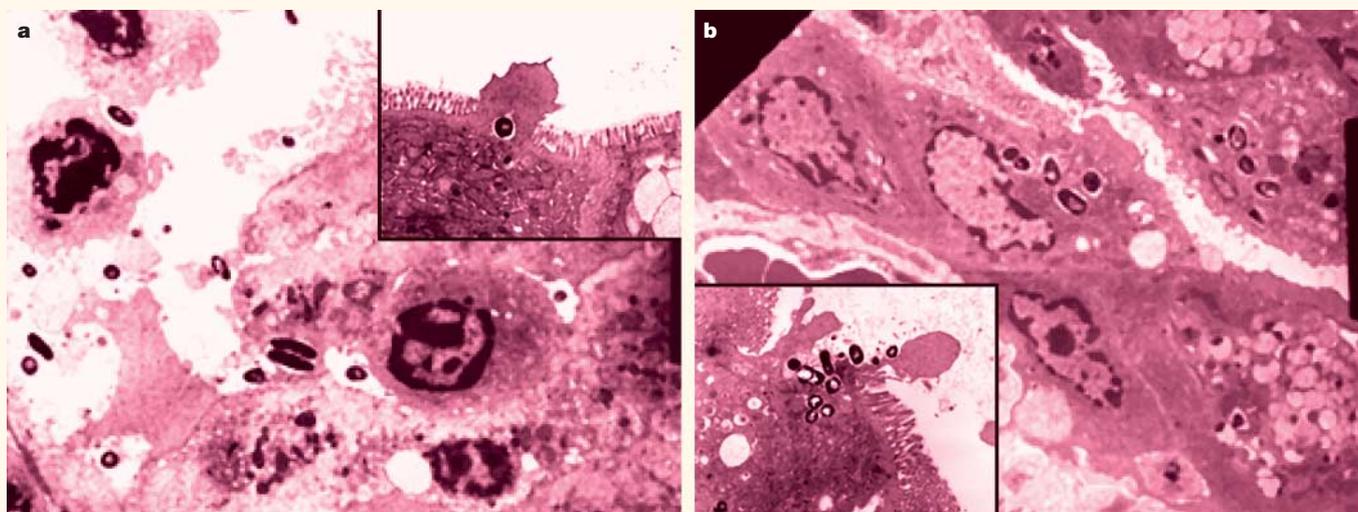
Figure 1 | **The use of gene knockout animals to help determine virulence gene function.** After oral infection, *Salmonella enterica* serovar Typhimurium SL1344 bacteria enter wild-type mice and congenic caspase-1 knockout mice identically through the Peyer's patch (see inset to FIG. 1a,b). However, after only an hour the bacteria that entered a wild-type mouse have caused significant cell death, and the bacteria are seen extracellularly among the disrupted epithelium (**a**). By contrast, in caspase-1 knockout animals, the bacteria are exclusively intracellular, and subsequently are almost completely eliminated by the host phagocytes (**b**). This difference shows that, in these animals, a single virulence gene, *sipB*, an effector of the SPI1 pathogenicity island cannot find its substrate, caspase-1, and cannot induce cell death of the host phagocytes that salmonellae initially encounter in the host's Peyer's patch. In this model, caspase-1 mice are >100-fold more resistant to oral challenge, although they are of unchanged susceptibility following intraperitoneal challenge. Reproduced with permission from REF. 18 © (2000) Rockefeller University Press.

phase of the infection. Mutations in SPI1 are attenuated for oral infection; however, SPI1 is not necessary for the systemic phase of the infection because SPI1 mutants are not attenuated when they are administered in mice intraperitoneally, which bypasses the gastrointestinal phase of the infection[18]. In macrophages, one SPI1-encoded effector protein, SipB, induces host-cell death through its interaction with a host cysteine protease, caspase-1 (REFS 19,20). Caspase-1 is a proinflammatory enzyme, and its activation by SipB not only induces cell death, but also leads to caspase-1 cleavage of the inactive precursors of interleukin (IL)-1β and IL-18 to generate the mature proinflammatory forms. Therefore, *Salmonella* kills the phagocytic cells it encounters but, paradoxically, at the same time 'deliberately' induces an inflammatory response that is destined to bring even more phagocytic cells into its immediate vicinity.

A direct role for SipB in SPI1-mediated cell death is supported by a number of observations. However, single mutations in the *sipB* gene do not lead simply to the loss of the ability to induce caspase-1 and cell death, but also to a loss of all SPI1 functions — presumably because disruption of the SipB protein leads to disruption of the mechanism by which effector molecules are translocated to host cells. So, although SipB, has been proposed to be the main activator for caspase-1 during *in vivo* infection, it has

been difficult to directly address this possibility owing to the multiple roles of SipB, in addition to directly activating caspase-1 (reviewed in REF. 19).

The use of a caspase-1-knockout mouse has provided a useful view of the role of SipB–caspase-1 activation in salmonellosis. Instead of inactivating the bacterial gene to accommodate the molecular Koch's postulates, Monack and co-workers used animals that had lost the host substrate for the bacterial gene[18]. Infection of caspase-1 knockout mice with wild-type *Salmonella* gives virtually the same phenotype as infection with a SPI1 or a *sipB* mutant. A >100-fold higher oral dose of *Salmonella* is required to lethally infect caspase-1 knockout mice compared with wild-type mice.

A microscopic and microbiological comparison of the fate of wild-type bacteria in normal mice and in caspase-1 knockout animals also reveals some interesting insights into *Salmonella* pathogenesis in the mouse model of infection and disease (FIG. 1). The bacteria enter the M-cells of caspase-1 knockout mice normally; however, within an hour of infection, the bacteria in the caspase-1 knockout mice are visible in host cells that seem to be macrophages or dendritic cells. By contrast, in wild-type infection of normal mice, there is clear evidence of host-cell death. The bacteria, rather than being intracellular, are most frequently seen in the extracellular spaces or in incompletely lysed

host-cell membranes. By three hours post-exposure in the capsase-1 knockout mice, there is essentially no sign of viable *Salmonella* microscopically, and there are lower numbers of *Salmonella* compared with wild-type mice, as monitored by culture. At this time-point, however, there is clear evidence of an acute inflammatory response in wild-type infection of normal mice. The bacteria are clearly present in cells resembling macrophages, and are viable and increasing in number. It is important to note that when the caspase-1 knockout mice are infected intraperitoneally, they respond in the same way as wild-type animals to *Salmonella* infection.

So the importance of caspase-1 and, it is supposed, the SipB protein (and other SPI1 effectors), seems to be restricted to the first phase of infection that involves crossing the intestinal barrier. One important role of SPI1 effectors seems to involve neutralization of the first line of phagocytic defence. I would further speculate that the subsequent provocation of an inflammatory response is required to bring in a specific cell-type, within which the SPI2 pathogenicity island is important to ensure the spread of the bacteria in the tissue[21]. It might be noteworthy that certain immune cells, such as dendritic cells and polymorphonuclear neutrophils that are attracted to the site of inflammation, might provide *Salmonella* with an intracellular niche and transportation from the Peyer's patch to adjacent lymphoid tissue sites[22]. Indeed,

inflammatory signals that are produced by caspase-1, especially IL-1β, are potent stimuli for dendritic-cell maturation and migration. The caspase-1 knockout mouse example provides a striking instance of how specific defects in the host can be used productively to examine the role of specific bacterial determinants of infection. As an aside, the host cells that are harvested from knockout animals can also be of considerable use in pathogenesis studies. For example, macrophages that are harvested from caspase-1 knockout mice allow the biology of the *Salmonella*–macrophage interaction to be examined in the absence of early induced host-cell death. Similar insights into other aspects of *Salmonella* infection have been obtained with other knockout strains of mice (reviewed in REF. 16). The main impact of such studies comes from examining defined bacterial mutants in defined host mutants, so that a study truly concentrates on a single bacterial determinant in a mutated host background containing a single mutation.

Host resistance loci identified by positional cloning of mouse mutations. Differences between inbred strains of mice in their susceptibility or resistance to a certain pathogen can be mono- or multigenic. A monogenic inheritance allows a positional cloning approach to identify the locus responsible, which has been used successfully to identify many spontaneous or induced mutations in the mouse[16]. One of the best-known examples of this approach is the identification of a gene expressed in macrophages that encodes an integral membrane phosphoglycoprotein of 90–100 kDa, which is known as NRAMP1 (natural resistance-associated macrophage)[16,23]. This gene is responsible for resistance in mice to several phylogenetically unrelated intracellular pathogens. Historically, most of the animal experiments that have been performed to study *Salmonella* pathogenesis have concentrated on animals that were not NRAMP1 proficient.

Most mouse studies of salmonellosis have used hyper-susceptible animals that have been challenged by a systemic (intravenous or intraperitoneal) inoculation route, which is not the usual oral route that occurs in nature. This reflects the fact that the use of NRAMP1-proficient mice required very large inocula of wild-type bacteria to achieve infection and disease. Furthermore, we have found that the infection of NRAMP1-proficient animals by the oral route can lead to asymptomatic infection, and very often to a prolonged carrier state in which the bacteria are found intracellularly within macrophages. In the carrier state, *Salmonella* are found

invariantly in the mesenteric lymph nodes and can sometimes be found in other organs as well[24]. A comparison of congenic NRAMP^{+/+} and NRAMP^{–/–} animals infected with wild-type or mutant bacteria and of cells harvested from these animals, will undoubtedly lead to discoveries of new bacterial virulence genes that are important for bacterial persistence in host animals. Indeed, these comparisons might provide information about suspected bacterial virulence genes that fail to give a credible phenotype in animal models of acute infection, but which are essential for asymptomatic persistent infection. Such genes will not fulfil the current molecular Koch's postulates, but they would be characterized, in my view, as pathogenicity genes in the context of the biology of the natural pathogenesis of salmonellosis, which depends on a carrier state that serves as the reservoir of infection in natural populations.

Another example of a host-resistance gene that was identified by a positional-cloning approach in mice is the *Tlr4* (Toll-like-receptor 4) locus, which acts as a host defence mechanism to detect minute quantities of bacterial lipopolysaccharide and responds to microbial invasion. Several more Toll-like receptor genes have been identified in mammals (for reviews, see REFS 6,7). Future studies will undoubtedly investigate their function in mediating a differential innate immune response to pathogens. Although the Toll-like factors have been characterized as detecting pathogen-associated motifs[6,7], owing to genetic-cloning studies in animals it is now clear that most, in fact, detect what are more aptly characterized as prokaryotic-associated motifs. The Toll-like receptors, therefore, signal to the host that its mucosal barriers have been breached by a microorganism, regardless of its intrinsic pathogenicity. Since most pathogens evolved in the presence of these innate immune factors (I assume that professional pathogens took the innate system as a given as they evolved), it could be that we will discern how specific pathogen virulence genes were evolved to defeat these stalwarts of the host immune defence system.

Finally, I should note here that another host-genetic approach to understanding the genes that are associated with host defence involves the selection of different inbred strains of mice that are susceptible or resistant to certain pathogens and the identification of complex gene interactions by QTL mapping. For example, Sebastiani and coworkers[25] studied the genetic basis of both resistance and susceptibility to infection with *S. typhimurium* in an inbred mouse strain (MOLF/Ei) and identified additional

resistance and susceptibility loci that are different from the previously identified *Nramp1* and *Tlr4* loci. They then mapped the *Tlr5* gene in the genetic interval of the identified *Salmonella*-susceptible QTL[26].

The QTL approach is interesting because it begins with genetically heterogeneous systems, not unlike that seen naturally in humans and other animals, and finds individual susceptibility or resistance genes for infection. This approach, as well as those mentioned above, provides the tools to evaluate specific host defects and to measure them in the face of infection with specific microbial mutants. This provides a more precise view of the host–pathogen interactions and provides a new level of sophistication in a molecular approach to the study of bacterial pathogenicity. When we discover that a single bacterial virulence gene impacts a defined host gene product or, at least, a single host pathway, it can be argued that we fulfil molecular Koch's postulates in every sense of its intent.

A virulence gene cannot be defined only in the context of the microorganism. Rather, microbial pathogenicity must be seen in the context of the host because a pathogen for one host might be only a transient in another or, at best, an opportunistic pathogen. Also, the pathogen might exhibit a different pattern of infection or disease in different hosts. This is certainly true in the case of *S. typhimurium*, which in wild-type animals establishes a systemic infection that leads to a significant number of asymptomatic carriers in mice, while causing a self-limiting diarrhoeal disease in humans. We have yet to understand the 'rules of deployment,' but I dare say we will gain considerable insight from the precise study of single virulence determinants in a defined host background that allows us to examine distinct biochemical pathways.

Using DNA microarrays

It is not possible to make knockouts of every host gene, or to manipulate host genes easily when they have an essential role in the biology of the host animal. However, this does not mean that one cannot monitor the precise effect of a specific bacterial virulence gene on the host-cell response to infection or disease.

Complete genome sequences of microbial pathogens and their hosts offer sophisticated new strategies for studying host–pathogen interactions. DNA microarrays exploit primary sequence data to measure transcript levels for every gene of a particular organism. In principle, this can be done simultaneously for both the host and the pathogen if the sequences are known and, in principle, it can be performed on the same experimental
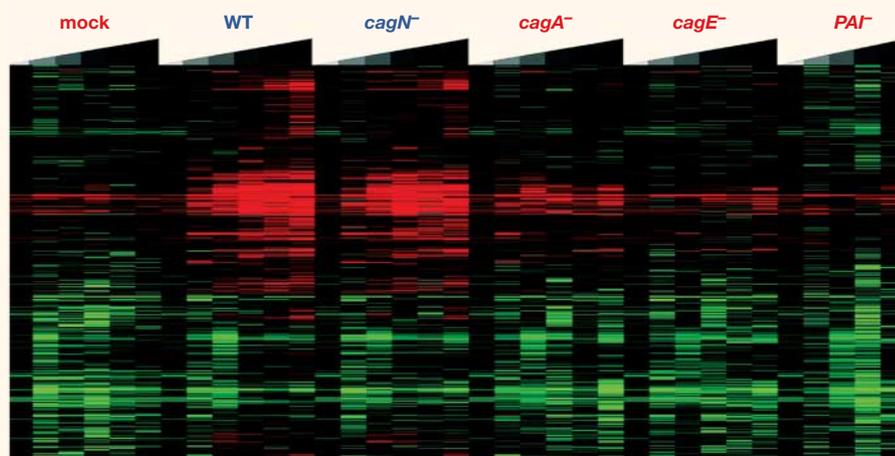
Figure 2 | **The use of the host response to infection to define the function of bacterial virulence genes.** Cultured human gastric epithelial AGS cells were infected with wild-type and well-defined mutants of *Helicobacter pylori* and at various times after infection, the transcriptional response of the host was measured using a 22,000 gene-element DNA microarray. The difference in the host-cell response to bacteria specifically lacking the bacterial virulence gene *cagA* compared with wild-type and other mutant *H. pylori*, showed that elements of the host tight junction were particularly associated with the presence of *cagA*, and this association was subsequently confirmed. Reproduced with permission from REF. 29 © (2002) National Academies of Sciences.

sample taken from an animal or cell-culture model of infection.

In cases where a known or suspected virulence gene is overexpressed or observed during infection of host cells, there has been considerable progress using DNA microarrays of bacterial genomes to compare the transcriptional response of wild-type and mutant bacteria under growth conditions that mimic those that are seen in the host[27]. In such studies, a large fraction of the genome can be simultaneously interrogated, and clustering of the data has helped to identify groups of genes that implicate activation or repression of key regulatory pathways, as well as unsuspected co-regulated genes that might contribute to pathogenicity.

Microarrays promise to accelerate our understanding of the contribution of the host in the host–pathogen interaction. We can follow the temporal sequence of transcription induction and repression, and improve our understanding of the order of events after a host–pathogen encounter. One important caveat to studying transcription in any system is that post-transcription regulatory events cannot be detected. This is particularly important in the case of the host response because many important host-cell events, such as cytoskeletal rearrangement and immune signalling, occur after transcription. Also, the effect of microbial infection might, as noted above, affect profoundly the physiological state of the host cell. Is the effect of infection on

the host cell, particularly apoptosis and necrosis, a primary or secondary product of an interaction with a pathogenic microorganism? Can we use genome-wide profiling to separate these primary and secondary effects?

A number of studies have examined some key aspects of the molecular programme of gene expression after infection by wild-type bacteria. For example, transcription profiling of macrophages and epithelial cells infected by *Salmonella* confirmed an increased expression of many proinflammatory cytokines and chemokines, signalling molecules and transcription activators, and identified several genes that previously had not been recognized as being regulated by infection. In macrophages, exposure to purified *Salmonella* lipopolysaccharide resulted in a response profile similar to that generated by exposure to whole bacterial cells[28]. The difficulty seen in these studies, as well as in a number of studies of the host-cell response to infection by pathogens, is that the innate immune response is rapidly engaged, and the host response that might be pathogen specific is overwhelmed by this common host-cell response. However, as in the case of classical genetic and biochemical studies, if the response of cells (or even of infected animal tissue) is compared with infection by wild-type and specific mutant bacteria, the response of the host to a defined virulence factor or to a block of functionally related genes, such as a virulence plasmid or a pathogenicity island can often be isolated.

As an example of the utility of this approach, Guilliman and co-workers recently examined the response of cultured gastric epithelial cells to *Helicobacter pylori* wild-type and mutant strains[29] (FIG. 2). *H. pylori* strains that cause ulcer disease and gastric cancer are more likely to have a pathogenicity island that encodes a type IV secretory system and the effector protein, CagA[30]. CagA is known to be inserted into host cells where it is phosphorylated on one or more tyrosine residues by a host-cell kinase. Subsequently, the host cells elongate, presumably as a consequence of the activation of the host-cell tyrosine phosphatase, SHP-2 (REF. 31). How CagA might mediate these effects is not clear. A comparison of the host-cell response to wild-type bacteria and to specific bacterial mutants defective in the CagA protein or in CagA translocation into host cells, revealed that there was no difference in the innate immune response induced by the different strains. However, compared with the wild-type strains, the *cagA* mutant strains failed to induce a significant number of host cytoskeletal genes and, particularly, did not upregulate representative genes of the tight junctional complex[29]. To study the cellular effects of CagA delivery to polarized epithelia with well-developed tight junctions, Amieva and co-workers established a model of chronic *H. pylori* cellular infection of Madin–Darby canine kidney (MDCK) cells[32]. CagA delivery to polarized epithelia leads to formation of ectopic patches of junctional proteins at sites of bacterial attachment, defects in barrier function and dramatic changes in cell morphology that exemplify a loss of cell polarity and cytoskeletal control. Our data indicate that CagA is able to co-localize and co-fractionate with the tight junction scaffolding protein ZO-1, and also with the transmembrane protein JAM.

Of course, these data do not allow us to understand the precise function of CagA, but the approach did provide us with an unexpected view of CagA function that is now amenable to further biochemical and genetic study. Despite the strong epidemiological and biochemical data that CagA is important for *H. pylori* disease, there is little or no difference in the effects caused by CagA-positive and -negative strains in cell culture and animal infection models, nor is the induction of malignancy clearly associated with CagA in animal models of disease. Therefore, CagA can be considered to have 'failed' molecular Koch's postulates. Yet the application of more refined tests of the specific effects of CagA on host cells provides a means to re-examine the impact of CagA on infected hosts.

I note here, in passing, that the method of RNAi provides us with a powerful alternative to gene-knockout mice, and works transiently so that even essential host genes can be targeted[32–34]. RNAi will undoubtedly be used in the future to investigate the finer details of the interactions of specific virulence determinants with host cell pathways in cell-culture models of infection.

Concluding remarks

Molecular Koch's postulates, even after fifteen years, might still have some use even though the study of pathogenicity at the genetic and molecular level has become increasingly refined. 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. Perhaps it is surprising that there is still no consensus about what is, or for that matter, what is not, a pathogen. In part, I think this reflects the residual distinction about whether one studies pathogenicity from the practical and necessary standpoint of the disease the microorganism might cause, or whether one takes the more academic view and is interested in the biology of the adaptation and evolution of the microorganism to the host without regard for the implications for diagnosis, treatment or prevention of disease. In some ways, the main conundrum to the study of pathogenicity has been the uncomfortable fact that many (most?) of the pathogens to which humans are susceptible show two faces.

Our interactions with a number of common aetiological agents of bacterial disease often results in asymptomatic carriage more frequently than frank clinical symptoms. The balance seems more often due to the failure of the host than to an encounter with organisms that are more virulent. The reservoirs of our most common, and often most deadly infections, are persistently infected asymptomatic individuals. We are only now understanding how this might occur and what constitutes pathogen manipulation and what constitutes host adaptation to a foreign incursion. The original molecular Koch's postulates were useful, I think, for establishing the elements of acute infection, such as adherence, toxins, invasion and avoidance of the innate defences of the host. We are only now coming to grips with bacterial virulence genes that are associated with transmission, the tactics of the microorganism to directly manipulate the host defences, and how microorganisms can persist for years, or even a lifetime, in the face of a robust immune response. The shared properties of commensals and of pathogens of the same host species can be extraordinarily close and a source of consternation when trying to define a pathogen in the medical sense versus the definition on purely biological grounds.

I offer no new postulates or rewording of the original postulates to reflect our new knowledge. The molecular Koch's postulates were not intended to be anything more than a means to provide a basis of dialogue among interested investigators. In this article, I have tried to highlight that the dialogue among investigators now takes on less of a phenotypic description based on only a few, often observational, criteria. The dialogue about bacterial virulence genes now centres increasingly on better defined biochemical mechanisms that are less equivocal. What is perhaps the most interesting turn of events, in my mind, is just how much we can use the host response to tell us about the function of bacterial virulence genes. In the future, we can expect, I think, to understand to an extraordinary degree the bacterial component of the host–pathogen interaction. And some day, I suppose, we all can agree about the fundamental idea that was inherent in the molecular version of Koch's postulates[36], 'what is a pathogen?'

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Competing interests statement
The author declares that he has no competing financial interests.