

## PREFACE

Pathogenic bacteria, i.e. those that produce disease, have unique biological properties, which enable them to invade a host and produce sickness. The molecular bases of these biological properties are the determinants of pathogenicity and research objectives are to recognize them, identify them chemically and relate their structure to function. Most of our present knowledge comes from studies with cultures *in vitro*. However, there is a rising interest in bacterial behaviour in the infected host and new methods have been developed for studying it. The objective of the Discussion Meeting was to describe these methods and to show how they, and a recent surge in conventional studies, are shedding light on the activities of bacterial pathogens *in vivo*. Participants were asked to enquire about bacterial and host factors that operate *in vivo* to bring about sickness, to show how phenomena recognized *in vitro* relate to behaviour *in vivo* and, if evidence of relevance is not available now, to indicate how it might be obtained.

There are two introductory papers. The first, by Smith, outlines the new methods, poses questions about the behaviour of bacterial pathogens *in vivo* and indicates how answers may be obtained. Growth *in vivo* and the underpinning processes of nutrition and metabolism are given special emphasis because new methods are highlighting their importance. The second, Marshall *et al.*, describes how the cellular environment can affect gene expression. It deals with the expression of genes coding for determinants of DNA topology (DNA gyrase, integration host factor and the nucleoid-associated protein H-NS) during adaptation of *Salmonella typhimurium* to the intracellular environment of macrophages. These global systems influence the transcription of genes involved in virulence, e.g. the *spv* locus. Next, five papers describe the new methods and their use in understanding host/pathogen interactions. The first, by Philpott *et al.*, shows how a combination of studies with cell cultures and those with various animal models (infections of macaques, rabbit intestinal loops and murine lungs) have defined the molecular basis for mucosal invasion and the stimulation of inflammation by *Shigella flexneri*, which may apply to dysentery in man. In the second, Merrell & Camilli describe the use of recombinase-based *in vivo* expression technology (IVET) to detect genes that are transcriptionally

induced during infection, including those expressed transiently or at low levels. Spatial and temporal expression of specific genes, e.g. for the toxin co-regulated pilus (*tcpA*) and cholera toxin (*ctxA*) can be monitored during the course of infection. Hautefort & Hinton discuss many techniques, other than IVET, for detecting gene expression *in vivo*, e.g. differential fluorescence induction and *in vivo* antigen technology (IVIAT). Some of these approaches can determine whether genes are expressed constitutively or in an organ-specific or cell-type-specific fashion. The paper by Unsworth & Holden describes signature-tagged mutagenesis and its use for *S. typhimurium* in a mouse model to identify many virulence genes required for growth *in vivo*, including several clustered on a chromosomal pathogenicity island. It also shows how the use of a temperature-sensitive, non-replicating plasmid and competitive index tests can demonstrate that virulence gene function *in vivo* may differ from that predicted from *in vitro* studies. Finlay & Brumell describe the interaction of *S. typhimurium* with relevant host cells both *in vitro* and in various animal models. Sophisticated imaging and molecular genetic tools are being used to monitor gene expression in both the pathogen and the host cell during infection. Tissue culture results have been confirmed and new questions evoked.

Three papers discuss the impact of the new methods. The first of these, by Heithoff *et al.*, describes identification by IVET of many housekeeping and virulence genes of *S. typhimurium*, which are induced only *in vivo*. Some of these genes are expressed *in vitro* if regulatory genes of the DNA adenine methylase system (Dam) are mutated. Dam-negative mutants illustrate how the loss of a single enzyme can completely block the ability of a pathogen to cause disease yet fully elicit a protective immune response. The paper by Moxon & Tang shows how a combination of genomics and methods for detecting gene expression *in vivo* are identifying genes that relate to virulence. It discusses practical and semantic difficulties in distinguishing between classical virulence factors and those that promote survival and growth in the host. It underlines the problem of obtaining animal models that reflect disease in the natural host. The paper by DiRita *et al.* relates knowledge of virulence gene regulation gained from studies *in vitro* to what occurs *in vivo* for two pathogens. For *Vibrio cholerae*, the ToxR regulon is active *in vivo* but the environmental factors that activate it are not clear. For *Streptococcus pyogenes*, capsule production occurs in an animal model of necrotizing skin infection. It is critical for virulence but dependent on

mutation in a two-component regulatory system CsrR and CsrS, i.e. on the loss of the regulation that occurs *in vitro*.

The next three papers discuss important aspects of bacterial pathogenicity and the evidence for their operation *in vivo*. Williams *et al.* describes quorum sensing, i.e. the regulation of bacterial processes in a cell-density-dependent manner through cell-to-cell communication by signalling molecules. Relevant signalling molecules have been detected in animal models and human infections. These molecules not only control bacterial gene expression but can also modulate host-cell responses. The paper by Cornelis describes the Yop virulon of *Yersinia* species as an archetype for type III secretion systems, which are activated by contact with eukaryotic cells. They allow bacteria to inject their proteins across two bacterial membranes and the host-cell membrane to destroy or subvert target cells. Studies with macrophages are described, but proof that they operate during animal infections has not yet been obtained. Morschhauser *et al.* shows that point mutation, genetic rearrangements and horizontal gene transfer processes contribute to macroevolution, long-term processes leading to new species, and microevolution, short-term developments occurring in days or weeks. Microevolution occurs *in vivo*; genome variability of pathogenic microbes leads to new phenotypes, which are important in acute development of an infectious disease. Horizontal transfer *in vivo* of genes by plasmids, bacteriophages and pathogenicity islands is more important for macroevolution.

The final paper, by Dougan *et al.*, raises practical implications of the new knowledge. It deals with the handling of mucosally delivered antigens in attempts to design effective vaccines. Studies are needed of the mechanism of pathogenicity employed by microbial pathogens, of the combined mucosal and systemic immune response associated with infection and recovery, and of the mechanism of action of known good mucosal immunogens. The importance of studies in the natural host or whole animal systems is emphasized.

Some important aspects that emerged during the Meeting are summarized. Many of the new methods for studying bacterial behaviour *in vivo* require the pathogen to have robust genetics capable of easy manipulation. This is not always so, for example for *Campylobacter jejuni*, and it is fortunate that some methods, such as IVIAT, can be applied to such pathogens. The current surge in knowledge of bacterial behaviour *in vivo* comes as much from application of conventional methods (chemical and biological

comparison of *in vivo*- and *in vitro*-grown organisms, mutation, virulence tests and complementation) as from new methods. A serious problem in applying both the new and conventional methods is the frequent lack of realistic animal models for human infections. This may severely limit our ability to get to the molecular basis of pathogenicity in humans. The problem may be mitigated in the future by the use of transgenic animals and the design of non-invasive methods for possible use in humans. Even when satisfactory animal models are available, better methods are needed for following the progress of infection spacially and in real time *in situ*. Because of its convenience, infection of macrophages in culture has been used as a halfway house between *in vitro* and *in vivo* conditions. Although these experiments may not reflect all the nuances of infection in animals, much useful information has been obtained from them, some of which has been confirmed by experiments with animals. At present, attention is largely concentrated on bacterial activities *in vivo* rather than the host factors that affect them and the interaction between the two. Most references to host factors are made in relation to the environment in macrophages or animals as a whole, rather than to specific factors and their changes during infection.

In the future, host DNA microarrays may be used to investigate global changes in eukaryotic gene expression in response to bacterial infection. Bacterial pathogens and their exoproducts are excellent probes for host cell biology. Some of the assumptions about the behaviour of pathogens *in vivo* based on research *in vitro* have been confirmed, particularly with regard to virulence determinants and regulatory systems. However, other assumptions have been shown to be too simplistic, e.g. operation of the ToxR regulatory system. Many of the genes expressed *in vivo* detected by the new methods are involved with nutrition, growth, metabolism and survival in the tissues of the host. Some well-known traditional virulence determinants — aggressins and toxins — have not been detected. Pathogens that are fully host adapted employ slip-strand mispairing to generate population diversity and have fewer transcription regulators than pathogens with both host and environmental lifestyles. We have moved from an era of the gene to the era of the genome and can now undertake ‘top-down’ approaches to problems of pathogenicity. Application of the new knowledge to the design of novel approaches to preventive therapeutic medicine has begun and will accelerate. Ways of inducing pathogen attenuation rather

than death may be derived. Many of the genes detected by the new methods are not known to be involved in metabolism, stress response, regulation or virulence-determinant production by the pathogen under consideration, nor of any other bacterial pathogen. Thus, vast areas of the behaviour of pathogens *in vivo* remain unexplained. A major challenge for the future will be integration of the vast amount of information that will accumulate from genomics with equally voluminous data derived from intensive use of the new methodologies for studying bacterial behaviour *in vivo*.

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