

Introduction

QUESTIONS ABOUT THE BEHAVIOUR OF BACTERIAL PATHOGENS *IN VIVO*

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Bacterial pathogens cause disease in man and animals. They have unique biological properties, which enable them to colonize mucous surfaces, penetrate them, grow in the environment of the host, inhibit or avoid host defences and damage the host. The bacterial products responsible for these five biological requirements are the determinants of pathogenicity (virulence determinants). Current knowledge comes from studies *in vitro*, but now interest is increasing in how bacteria behave and produce virulence determinants within the infected host. There are three aspects to elucidate: bacterial activities, the host factors that affect them and the metabolic interactions between the two. The first is relatively easy to accomplish and, recently, new methods for doing this have been devised. The second is not easy because of the complexity of the environment *in vivo* and its ever-changing face. Nevertheless, some information can be gained from the literature and by new methodology. The third aspect is very difficult to study effectively unless some events *in vivo* can be simulated *in vitro*.

The objectives of the Discussion Meeting were to describe the new methods and to show how they, and conventional studies, are revealing the activities of bacterial pathogens *in vivo*. This paper sets the scene by raising some questions and suggesting, with examples, how they might be answered.

Bacterial growth *in vivo* is the primary requirement for pathogenicity. Without growth, determinants of the other four requirements are not formed. Results from the new methods are underlining this point. The important questions are as follows. What is the pattern of a developing infection and the growth rates and population sizes of the bacteria at different stages? What nutrients are present *in vivo* and how do they change as infection progresses and relate to growth rates and population sizes? How are these nutrients metabolized and by what bacterial mechanisms? Which bacterial processes handle nutrient deficiencies and antagonistic conditions that may arise? Conventional and new methods can answer the first question and part of the second; examples are described. The difficulties of trying to answer the last two are discussed.

Turning to production *in vivo* of determinants of mucosal colonization, penetration, interference with host defence and damage to the host, here are the crucial questions. Are putative determinants, which have been recognized by studies *in vitro*, produced *in vivo* and are they relevant to virulence? Can hitherto unknown virulence determinants be recognized by examining bacteria grown *in vivo*? Does the complement of virulence determinants change as infection proceeds? Are regulatory processes recognized *in vitro*, such as ToxR/ToxS, PhoP/PhoQ, quorum sensing and type III secretion, operative *in vivo*? What environmental factors affect virulence determinant production *in vivo* and by what metabolic processes? Examples indicate that the answers to the first four questions are 'yes' in most

but not all cases. Attempts to answer the last, and most difficult, question are also described.

Finally, sialylation of the lipopolysaccharide of gonococci *in vivo* by host-derived cytidine 5'-monophospho-N-acetyl neuraminic acid, and the effect of host lactate are described. This investigation revealed a new bacterial component important in pathogenicity, the host factors responsible for its production and the metabolism involved.

Keywords: bacteria; pathogens; *in vivo*; gonococci; sialylation; lipopolysaccharide

1. INTRODUCTION

Pathogenicity (virulence) is the capacity to cause disease. Bacterial pathogens form only a small part of the bacterial world but they attract the most attention. They have unique biological properties, which enable them to enter the tissues of man or animals and cause sickness and sometimes death. These biological properties are indicated by the progression of the disease process. The skin is a formidable barrier against bacterial attack. It can be breached by gross trauma or vector bite and a few pathogens, e.g. staphylococci are able to exploit small abrasions to cause skin infections. However, the usual route of entry for most pathogens is not through the skin but over the internal surfaces of the respiratory, alimentary or urogenital tracts. Initially, only a small number of bacteria are deposited on these mucous surfaces, so the first requirement for pathogenicity is survival and growth on them. Survival entails competition with commensals that normally inhabit these surfaces, penetration of mucus that covers them and adherence to epithelial cells. Next, most pathogens need to penetrate into the tissues to be effective, although some, like *Vibrio cholerae*, can cause disease while remaining on the mucous surface. Penetration may be achieved by entry into and egestion from epithelial cells, by passage between them or destruction of them. The third requirement is the ability to grow and multiply in the environment of host tissues, otherwise the pathogen cannot cause harm. On the mucous surfaces and within the tissues, pathogens have to contend with antibacterial substances in body fluids and within the cells they infect. Also, there are polymorphonuclear (PMN) phagocytes and macrophages that can ingest and kill them. These defence mechanisms, which act against any invading pathogen, are present at the site of infection and, within a few hours, are reinforced by the inflammatory response. Clearly, ability to withstand these host defences is the fourth requirement

for pathogenicity. This quality is needed again as bacteria spread through the lymphatic system into the bloodstream, where many macrophages line the vessels of the lymph nodes, spleen and liver. A few days after initial infection, the pathogen faces an even greater obstacle, the specific immune response. To survive, it must either suppress or circumvent antibody- and cell-mediated immunity. Finally, entry and growth in the host and inhibition of host defence are not enough. To be pathogenic, the bacteria must damage the host, thus causing disease. This can be achieved directly either by production of toxins or lysis of host cells by intracellular bacteria. It can also be accomplished indirectly by stimulation of host cytokines or immunopathology.

To summarize, for the many pathogens that do not enter the host by direct penetration of skin, there are five essential biological requirements for pathogenicity: colonize mucous surfaces; penetrate them; grow in the tissues; inhibit host defence; and damage the host. The cardinal fact about pathogenicity is that it is multifactorial. Many genes are involved. Their products, the determinants of pathogenicity (virulence determinants), are the molecular bases for the five essential biological properties (Smith 1995). The goal of studies on bacterial pathogens is to recognize these determinants, to identify them and to relate their structure to function.

In the past, most of our knowledge about these determinants has come from experiments with bacteria grown in culture. Almost all pathogens have been investigated and many determinants have been identified. Examples are the pili of gonococci, which aid mucosal colonization; the Ipa proteins of shigellae, which are involved in invasion of colonic epithelium; the enterochelin of *Esherichia coli*, which aids acquisition of iron for growth; the capsular polysaccharides of pneumococci that interfere with host defence; and the lethal toxin of diphtheria bacilli (Smith 1995; Finlay & Falkow 1997).

Now, the situation has changed. There is a burgeoning interest in the activities of bacteria within the infected host. Environmental conditions *in vivo* (osmolarity, pH, E_h , and nutrient and substrate availability) differ from those in laboratory cultures. They are more complex. Pathogens may be in films on mucosal surfaces or free in body fluids, cell cytoplasm or cell vacuoles; in all cases, the environment is neither simple nor defined. Also, the conditions change during the course of infection due to inflammation, tissue breakdown and spread from one anatomical site to another. Within

cells, the environment can alter when bacteria invade due to changes in host gene expression. Since environmental conditions affect bacterial growth, metabolism and regulation of gene expression (Busby *et al.* 1998; Marshall *et al.*, this issue) we should expect bacteria taken from infected animals to be different, in some respects, from those grown *in vitro*, a fact now well established for many pathogenic species (Smith 1990, 1996).

Clearly, the activities of bacteria *in vivo* must be explored for a fuller appreciation of pathogenicity. There are three aspects to the full picture. First, there are observations on the bacteria themselves and identification of virulence determinants formed by them at different stages of infection. Second, there is recognition of host factors that affect bacterial behaviour and production of virulence determinants. Third, there is investigation of the underlying metabolic interactions between bacterial and host factors. The first aspect is relatively easy to accomplish and recently new methods for doing so have been devised. The second is not easy to achieve because of the complexity of the environment *in vivo* and the fact that it changes as infection proceeds. Nevertheless, there is relevant information in the literature and some new methods have been evolved. The final aspect is very difficult to study effectively *in vivo*; some progress might be made if events *in vivo* can be simulated *in vitro*. It is not surprising that the first aspect receives most attention. Indeed, one can understand an attitude to concentrate solely on bacterial properties because the environment *in vivo* and its influence are too complex to analyse properly. However, this leaves part of the story untold.

The new methods for studying bacterial pathogens *in vivo* are listed in Table 1. Some require the pathogen to have robust genetics that are easily manipulated, which is not always the case, e.g. for *Campylobacter jejuni*. The objectives of this Discussion Meeting were to describe these methods and to show how they, and a recent surge in conventional studies, are advancing knowledge. This paper sets the scene by posing some questions and suggesting, with examples, methods whereby answers may be forthcoming.

2. QUESTIONS ABOUT THE DETERMINANTS OF BACTERIAL GROWTH *IN VIVO*

The multifactorial nature of pathogenicity means that the determinants of all five requirements are essential for its manifestation. However, growth