VIRULENCE MECHANISMS OF BACTERIAL PATHOGENS

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PREFACE

Generation of new ideas and refinement or extension of established concepts are the essence of advances in knowledge. The former occurs infrequently, requires broad vision, and has the potential to open up new vistas for examination. The role of bacterial toxins in disease, recognized in the late 19th century, is an example of such a novel idea. The critical role of bacterial adherence to mucosal surfaces is a more recent example of a new concept in pathogenesis which has had a significant impact on our understanding of disease processes due to bacteria. Another broadly based mechanism of disease is the possession by pathogens of systems which enable them to compete with animal hosts for scarce substrates such as iron. Also, host factors, particularly cell-mediated immunity and immunity at mucosal surfaces, have increasingly been recognized to be critical in the outcome of bacterial disease.

Once formulated, new principles in bacterial pathogenesis tend to generate an aura of excitement and an intense search for answers to new questions. Scientists are driven to establish how widely applicable the concept is, to determine variations on the theme that undoubtedly exist in nature, to purify and characterize the bacterial components involved, to identify the host factors implicated, to understand the genetic regulation of both bacterial and host factors, and to fill in missing details. Pursuit of these questions often leads to discoveries which, by themselves or taken with other information, form the basis of new concepts.

Traditionally, the study of bacterial virulence mechanisms has been dominated by individuals trained as bacteriologists or immunologists and with a medical or veterinary background. In recent years there has been a dramatic shift in the investigation of bacterial virulence. We now want to understand things at the molecular level and have the capacity to do this. It is no longer good enough merely to identify the gross and microscopic lesions in tissues. We need to know the biochemical lesion and to identify the specific host reactions that are impaired. Furthermore, we have come to realize that the powerful new tools of molecular genetics can be of immense assistance as we try to understand how bacteria cause disease. Transposon mutagenesis, recombinant DNA technology, gene cloning and sequencing, understanding the substrate and temperature conditions which regulate genes involved in virulence, and synthesizing DNA of interest and peptides of value are now common methods and approaches in the quest for understanding disease processes. The possibility of a new generation of vaccines and pharmaceutical agents has spurred on research on pathogenesis: if we understand how the bacterium causes disease and how the host responds to infection, our chances of selecting the best strategies for prevention and therapy are enhanced.

Given the new emphasis, it is not surprising that the field of pathogenesis in general and of virulence mechanisms in particular has been invaded by basic scientists, especially molecular biologists, and has been enriched by their presence. This development represents a challenge for the rest of us to bring the sophistication and precision of the basic scientists to bear on our own studies and to work

with these colleagues, because our combined skills can provide new insights.

Despite the unquestionable value of research at the molecular level, we need to ensure that deficits in information in areas beyond the interaction of host and pathogen at the molecular level are not ignored. To understand pathogenesis, we need to be fully informed about the habitat of the bacterium and the circumstances under which infection occurs. The biological context must not be lost amidst the glamor of the new technologies.

If we look continually at the same object under the same conditions, we lose the prospect of seeing anything new: our vision is framed by our limited experience and by our notion of what we expect to see. This book provides a unique opportunity for recognizing new perspectives on virulence mechanisms in bacterial diseases. Mechanisms of bacterial virulence do not respect the boundaries erected between humans and other animal species, and this volume brings together outstanding researchers who have looked at bacterial virulence from different vantage points and the experiences of a variety of disciplines: medicine, veterinary medicine, genetics, biochemistry, immunology, and microbiology. Although there are opportunities for examination of details, the big picture is still the overall theme: there can be no consideration of bacterial virulence without reference to the interaction of pathogen and host.

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Section I: Mechanisms of Bacterial Adherence, Colonization, and Invasion

therefore, attempts to provide a method based on a predictive supply model which can be used to provide relatively accurate and timely estimates at a nominal cost to any interested person or institution.

An idea about the nature of the present procedure of the estimation of crop production could be obtained by examining the magnitude of revisions made and the dates on which first estimate, final estimates (FE) and fully revised estimates (RE) are issued.[2] Table 1.1 provides dates of the first estimate and final estimate of area and it also provides first and final estimates of area and the final estimate of production for two years for illustration.[3] It is evident that even the first estimate, which is only the area estimate does not remain forecast because it is issued much after the crop starts coming to the market. For example, November is the month of the issue of forecasts for most kharif crops by which period the marketed supply has reached its peak, while final estimates which are based on cadastral surveys for area under crops and crop-cutting experiments for output, are issued at a time when off-season marketing takes place.[4] An examination of Table 1.1 further indicates that there is a wide difference between the first forecast and the final estimate of area. For example, in 1974-75 the difference was 41 per cent for groundnut and 31 per cent for other kharif pulses. For kharif foodgrain crops the difference was about ten per cent but for cotton and groundnut the difference was quite frustrating. Wheat and gram estimates, however, were quite reliable, but estimates of barley had substantial errors.[5] Thus, it is obvious that the first forecast of area is very unreliable for formulating and managing marketing policy. The final estimates, on the other hand, are available much later in the season or even after a year and therefore these are not useful to the procurement or price fixation agency.[6]

The following abstract from a review of the crop situation indicates that outlook for kharif foodgrains for the year 1976-77 was hazy even as late as November, 1976:

> It is *too early* to give a precise idea about the prospects of kharif crops (1976-77) at this stage...As per present indications the output of kharif foodgrains is likely to be *somewhat* lower than that of last year, whereas an increase is expected in the case of sugar cane, jute and cotton.[7] (emphasis added).

Chapter 1

Bacterial Infection of Mucosal Surfaces: an Overview of Cellular and Molecular Mechanisms

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INTRODUCTION

Bacterial colonization of a mucosal surface requires that bacteria (i) establish close proximity to the mucosa, (ii) avoid being swept away, (iii) acquire essential nutrients for growth, (iv) replicate at a rate sufficient to maintain or expand their population, and (v) resist local host defenses. Mechanisms by which bacteria maintain close proximity to a mucosal surface can be loosely categorized as association, adhesion, and invasion according to the degree of intimacy between bacterial and mucosal surfaces. Association, the least intimate form of surface interaction, implies weak, reversible attachment or localization of bacteria along a surface (Fig. 1). Adhesion, a more intimate form of attachment than association, describes relatively stable, irreversible attachment mediated by specialized complementary molecules of the bacterial and mucosal surfaces. The most intimate form of bacterial-mucosal interaction is invasion, wherein pathogenic bacteria penetrate the mucosal barrier to establish themselves within epithelial cells or adjacent stromal tissue. The purpose of this chapter is to review many of the cellular and molecular mechanisms of bacterial association, adhesion, and invasion within the context of mucosal colonization. Because many mechanisms are common to animal and human disease, an attempt is made to integrate some of the medical and veterinary literature that has contributed to our current understanding of bacterial infections of mucosal surfaces. The overview of bacterial colonization is followed by a discussion of virulence mechanisms of selected bacterial pathogens of the respiratory tract, ocular tissues, and skin.

COLONIZATION

Studies of bacterial adhesion and colonization were originated by marine and soil microbiologists in the 1930s and 1940s (86, 87). Early microbiologists used glass slides submerged in water or soil to collect and study adherent bacterial colonies (86). In 1940 Heukelekian and Heller found that nutrients, having a tendency to adsorb to and concentrate on

Table 1.1 (*Contd.*)

Crop	Date of estimates*		Year 1971-72			Year 1974-75		
	First	Final	Area estimates		Production estimates	Area estimates		Production estimates
			First	Final	Final	First	Final	Final
Gram	January	May	7.8	8.0 (102)	5.1	7.0	7.1 (101)	4.0
Barley	January	May	2.6	2.4 (92)	2.5	2.6	2.9 (111)	3.1
Groundnut	August	February	5.4	7.2 (133)	5.7	5.1	7.2 (141)	5.0
Cotton	August	May	5.8	7.8 (134)	11.7	5.7	7.6 (133)	12.2

*This information is taken from the *Handbook on Methods of Collection of Agricultural Statistics in India*, Directorate of Economics and Statistics, Ministry of Agriculture, New Delhi, 1959.

Notes: 1) Figures in brackets are percentage of final forecast as compared to first forecast.

2) Production of cotton is in bales of 180 kg.

Source: Agricultural Situation in India, different issues.

(Fig. 2). In contrast, the large intestine, ruminant forestomachs, vagina, uterus, and skin represent epithelial surfaces which lack highly efficient physical clearing mechanisms. Therefore, bacterial pathogens colonizing these tissues may rely less on specific adhesion and more on the weak, reversible interaction termed association (Fig. 1). Such bacteria may maintain association with the mucosal surface by binding to mucus or by chemotaxis.

Although mechanisms of colonization are emphasized below, it must be remembered that bacterial virulence usually requires multiple factors. For example, production of both enterotoxin and colonizing factors is required for pathogenicity of enterotoxigenic strains of *Escherichia coli*. Loss of a single gene product may prevent an otherwise virulent organism from colonizing its usual mucosal habitat. However, many bacterial pathogens have multiple fail-safe mechanisms to help ensure at least some level of colonization. Some strains of enterotoxigenic *E. coli*, *Pseudomonas aeruginosa*, and *Bordetella pertussis* produce several different surface-adhesive molecules which bind the bacteria to the host epithelium. The loss of one colonization factor may only reduce adhesive efficiency. Since many virulence factors are encoded by plasmid DNA, these bacteria have a grand repertoire of potential virulence factors available to ensure successful colonization. Our challenge is to characterize molecular mechanisms of colonization, devise strategies to disrupt colonization by pathogens, and yet cause minimal perturbation of the indigenous microflora and host tissue.

ASSOCIATION

Association is a nonspecific term for the localization of bacteria on a surface; it does not specify the mechanisms involved (133). The term is used in this chapter to describe the loose, reversible attachment or localization of bacteria in close proximity with a mucosal surface. Association may precede specific adhesion or invasion (Fig. 1). Bacteria may maintain their position along a mucosal surface by associating with mucus or exudates, by establishing small numbers of noncovalent bonds between the bacterial and mucosal surfaces, or by chemotaxis.

Chemotaxis is a significant virulence mechanism of bacterial pathogens of mucosal surfaces. Studies with *Vibrio cholerae* and *Sal-*

FIGURE 2. Bacterial colonization of the bronchial mucosa. Dense colonies of *B. bronchiseptica* are intimately attached to the cilia of bronchial epithelial cells in a young dog with kennel cough. The disease shares several similarities with whooping cough of humans.