Receptors and Recognition

Series B Volume 6

Bacterial Adherence

Edited by
E. H. Beachey

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Veteran's Administration Hospital, Memphis, Tennessee

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Preface

Bacteria adhere to and colonize almost any surface. Within minutes after submerging a solid object in seawater or freshwater, the surface becomes colonized by adherent micro-organisms, and the earliest organisms to adhere are bacteria. Adherent colonies of bacteria have also been observed on particles of sand, soil, other bacteria, plant tissues, and a variety of animal tissues. Shortly after birth, the skin and the mucosal surfaces of the upper respiratory tract and the gastrointestinal tract of animals and man become heavily colonized by a variety of adherent bacteria which persist in varying numbers as indigenous parasites. The apparent symbiotic balance between the host and his indigenous parasites occasionally is upset by the invasion of harmful bacteria which adhere to and colonize these surfaces. Pathogenic bacteria may also adhere to and colonize normally sterile surfaces such as the mucosa of the genito-urinary tract and the lower respiratory tract, and occasionally even endothelial surfaces of the cardiovascular system, resulting in the development of serious infectious diseases.

Although marine microbiologists have been aware for a long time that bacteria must stick to surfaces in order to avoid being swept away by moving streams of water, not until recently has it been widely recognized that adherence must be an important ecological determinant in the colonization of specific sites in plants and animals, and in particular an important early event in the pathogenesis of bacterial infections in animals and man. It is true that Dr G. Guyot as early as 1908 (*Abl. Bakt.*, I. *Abt. orig.* 47, 640) reported studies on the adhesiveness of bacterial cells for blood erythrocytes, and that some 20 years ago Dr Duguid (see Chapter 7) had already demonstrated the mannose sensitivity of the adherence of several genera and species of gram-negative bacteria to erythrocytes and intestinal epithelial cells. Nevertheless, the study of the mechanisms of bacterial adherence did not really 'catch on' until about 10 years ago when Dr R.J. Gibbons and his colleagues began reporting a series of elegant studies showing the selective nature of the adherence of bacteria to the various niches of the oral cavity and dental surfaces (see Chapter 3). Largely because of these studies, bacterial adherence has grown into one of the most active, if not the most exciting, areas of study in the field of microbial ecology and infectious diseases.

This book compiles for the first time into one volume a series of monographs prepared by invited experts on the subject of bacterial adherence. A particular emphasis is placed on the possible role of bacterial adhesive properties in the colonization and ecology of a variety of surfaces, both animate and inanimate. The mechanisms by which bacteria interact with these surfaces, be they smooth

surfaces or tissue cell membranes, are examined in detail, the implicit premise being that the interaction of bacteria with surfaces involves specific molecular ligands (or adhesins as some authors prefer to call them) on the surfaces of bacteria, which interact by a specific 'lock and key' mechanism with receptor molecules on the surfaces to be colonized. Moreover, it is presumed that the isolation and identification of the adhesive molecular structures on both surfaces will suggest new approaches to the control of certain bacterial infectious diseases. One obvious approach would be to try to block the adherence of harmful bacteria by the application of ligand or receptor materials once these substances or their analogues have been identified. Indeed, limited success has already been reported using such approaches and is discussed by several of the contributors to this volume.

The first ten chapters of this book are devoted to detailed studies of the chemical and molecular basis of the association of bacteria with animal tissues. The adhesive properties of marine micro-organisms are discussed by Fletcher in Chapter 11 and that of plant micro-organisms by Drs Lippincott and Lippincott in Chapter 12. This is followed by a more general view of recognition systems among eukaryotic cells elegantly discussed by Phillips and Gartner. In a more philosophical vein, Dr Freter, in the final chapter, discusses prospects for the future both with regard to the possible means one might use to interfere with the adherence process to prevent infectious diseases and with regard to the directions future work in the field should take.

It is hoped that this book will be of interest to a wide audience including microbiologists, physicians, dentists, cell biologists, biochemists, and many others. It is hoped in particular that the various contributions will serve to arouse the interest of students of the biological sciences to pursue specific problems which remain to be unraveled in this rapidly growing area of microbial ecology and infectious diseases.

As with most multi-author books, the present book on *Bacterial Adherence* has some unavoidable overlaps among chapters and in a few instances there may even be some duplication. However, it was considered more desirable to allow each chapter to stand on its own rather than to shorten the book by only a few pages.

My editorial duties on this book were made much easier by the untiring secretarial assistance of Mrs Johnnie Smith who among other things, gently prodded the contributors to submit their articles. My special thanks to Dr Uli Schwarz, who granted me beautiful laboratory and office spaces at the Max Planck Institute für Virusforschung in Tübingen, W. Germany, during a sabbatical leave from the Veterans Administration Hospital and University of Tennessee in Memphis. Many of my editorial duties on this volume were discharged while there. Many thanks to my longtime colleague and friend, Dr I. Ofek, for lively and stimulating discussions and for many helpful suggestions. The comments and suggestions offered by many of the contributing authors are also deeply appreciated.

May, 1979 Edwin H. Beachey, M.D.

Contents

1 General Concepts and Principles of Bacterial Adherence in Animals and Man

ITZHAK OFEK and EDWIN H. BEACHEY

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1.1 INTRODUCTION

Adherence of bacteria to tissue surfaces has recently gained increasing attention as an important initial event in the pathogenesis of bacterial infections (Ofek and Beachey, 1980). The infectious process in animals and man can be envisioned as a stepwise process in which bacteria must first adhere to a tissue surface. The failure to adhere would result simply in their being swept away in the fluids which constantly bathe the tissue surfaces. Adherence of pathogenic organisms is then followed by colonization and eventual invasion of the surface either by a toxin produced by the colonizing organisms or by the bacteria themselves. In the deeper tissues, the attachment of the bacteria to phagocytic cells results in their ingestion and destruction. Organisms whose surfaces are not recognized by antibody and complement or phagocytic cells can multiply unimpeded to produce systemic infections. These steps in the infectious process are depicted diagramatically in Fig. 1.1.

Although the fact that bacteria adhere to specific tissue surfaces has been well-documented, the precise role of adherence in tissue colonization and the development of infectious diseases has been difficult to define primarily because the interaction between bacteria and the various tissue surfaces represent cell–cell interactions which are physicochemically so complex that the specificity of the interactions are difficult to assess in a single model system. The study of a single modality such as adherence may give only partial clues as to the complex mechanisms of the interaction of bacteria with specific tissue surfaces in the intact host. For example, Freter (1978; see also Chapter 14, this volume) has pointed out that *V. cholera* infections of the gastro-intestinal tract involve a number of complex mechanisms including bacterial motility, chemotactic attraction, penetration of the mucus gel on the intestinal villi, adhesion to receptors in the mucus gel, chemotaxis into deeper intervillous spaces, adherence of the organisms to the epithelial surface and finally the elabotation of an injurious toxin.

Since the intitial cell–cell interaction is a surface phenomenon, investigators have been searching for molecules on the surfaces of the prokaryotic and eukaryotic cells that might attract and bind each other in a specific way. Thus far, most of these studies have been performed *in vitro*. Suspensions of eukaryotic cells (e.g. epithelial cells, erythrocytes, monolayers of tissue culture cells, excised tissues) are exposed to a standardized concentration of bacteria for a given period of incubation. The non-adherent bacteria are then removed by filtration or by repeated differential centrifugations. The advantage of using these *in vitro* systems is that one can readily assess the effects of various conditions of incubation or of various defined substances upon the adherence process. The disadvantage is that many of the physiological barriers of the intact tissue surfaces as outlined above are bypassed. Therefore, the

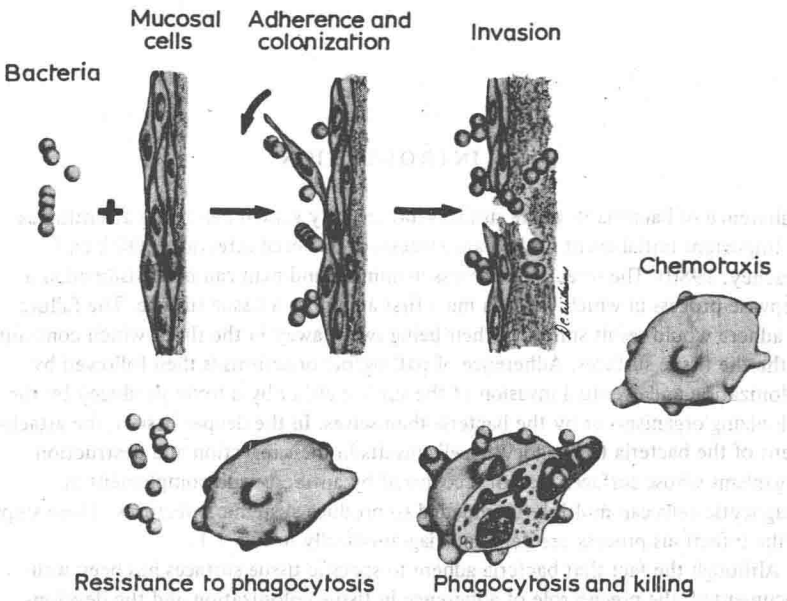

Fig. 1.1 Schematic of the proposed sequential steps in the infectious process in animals and man. In the first step, bacteria adhere to and multiply on the tissue surface, composed of epithelial cells in this diagram. In order to maintain colonization, the organisms must replenish the newly exposed epithelial cells as old cells with adherent bacteria are lost by desquamation (top center; see also Section 3.4 and Fig. 3.7). Injury to the epithelial cell barrier either by toxins produced by the bacteria themselves, or by other agents such as viruses, allows the bacteria to invade into deeper tissues (top right). In response to chemotactic stimuli produced by the invading organisms in conjunction with antibodies and complement, phagocytic cells (far right) migrate into the area. If the organisms lack surface components to mask their ligands, or if the host is immune and coats the bacteria with opsonic antibody, the organisms are recognized by receptors on the phagocytic cells for the bacterial ligand itself or for the Fc portion of the opsonic antibody, respectively. Once attached, the organisms are ingested and destroyed (bottom right). If, however, the organisms produce capsular material to which the host is not immune and which masks surface ligands, the organisms are not recognized by the phagocytic cells (bottom left) and are thereby able to multiply unhindered to produce a systemic infectious disease.

data obtained from *in vitro* experiments must be interpreted with caution as to their *in vivo* significance. Nevertheless, with this caution in mind, *in vitro* methods may be useful in identifying the molecules which cause the cells to stick to each other once they do come into

In this chapter we will deal with some of the general aspects of bacterial adherence with special emphasis on the epithelial cell. Epithelial cells cover all external surfaces of animals and humans and therefore represent the first cells that the colonizing organisms must encounter.

1.2 REPULSIVE AND ATTRACTIVE FORCES

The surface charges of eukaryotic and prokaryotic cells are, in sum, both negative. Yet there are both long-range and short-range attractive forces which act to overcome the repulsive force between the like-charged surfaces. The long-range attractive forces operating according to the DLVO theory are elegantly discussed by Watt and Ward in Section 9.5.2 and will, therefore, not be dealt with further here. Rather we focus on non-specific and specific short-range forces.

1.2.1 Surface hydrophobicity and net surface charge

Several methods have been developed to measure surface charge and surface hydrophobicity of bacteria in relation to adherence. Brinton (1965) measured the electrophoretic mobility of bacteria and found that the type 1 fimbriated *E. coli*, which adhere readily to cells, are less mobile than the non-fimbriated organisms which lack adhesive properties (see also Chapter 8, this volume). The importance of net surface charge was perhaps best demonstrated by Ward and his colleagues (see Section 10.5.1) who demonstrated that the adherence of non-fimbriated gonococci could be increased to the level of fimbriated organisms by chemically modifying the surface charges of the non-fimbriated bacteria. It is suggested that fimbriae increase adherence by simply counteracting repulsive electrostatic forces.

The hydrophobicity of the bacterial surface can be measured by partition of the bacterial suspension between biphasic aqueous solutions (Stendahl *et al.*, 1973), or by measuring the degree of adsorption of the test strain to hydrophobic gels consisting of Sepharose beads covalently linked to various hydrophobic residues (Smythe *et al.*, 1978). Perers *et al.* (1977) and Kihlstrom and Edebo (1976) found that the degree of hydrophobicity of *S. typhimurium* and *E. coli*, as measured by the partition method, correlated with the ability of the organisms to adhere to tissue culture cells. The greater the hydrophobicity the greater was the adherence. Smyth *et al.* (1978) found that K-88 positive strains of *E. coli* which agglutinate erythrocytes, adsorb strongly to hydrophobic ligands on Sepharose beads as opposed to the isogenic mutant which lacked K-88 antigen and did not agglutinate erythrocytes (see also Section 9.5.5).

It is possible that hydrophobic molecules on the bacterial surface allow the bacteria to approach the negatively charged epithelial cell and thereby enable special binding molecules on each of the cell surfaces to interact with each other to form specific bonds of high affinity (Fig. 1.2). By analogy, concanavalin A, which

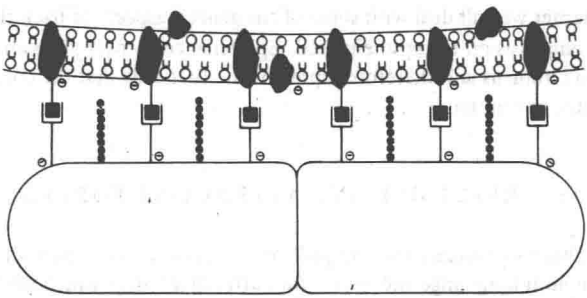

Fig. 1.2 Attachment of a bacterium (bottom) via specific ligands (Y) to complementary receptor (■) on the membrane (top) of an animal cell. In order to overcome the net negative charge (⊖) on both the bacterial and cell membrane surfaces, hydrophobic molecules (●) on the surface of the bacteria are attracted toward the hydrophobic phospholipid molecules (♋) in the lipid bilayer membrane. The irregular black structures represent protein and glycoprotein molecules incorporated into the animal cell membrane.

binds specifically to mannose residues, has been shown (Lis and Sharon, 1977) to possess hydrophobic sites which facilitate the interaction of the lectin with the membrane receptor. Once bacteria become attached to the cells, the avidity of attachment is dependent upon the formation of many bonds which, for practical purposes, are irreversible since the probability for all of the binding sites to become simultaneously unbound is very small (Bell, 1978).

1.2.2 Ligand–receptor interactions

There is much evidence to suggest that bacteria possess molecules on their surfaces capable of binding in a stereospecific fashion with complementary molecules on the surface of tissue cells of the host (Jones, 1977; Ofek *et al.*, 1978) (see Fig. 1.2). In this chapter, the binding molecules on bacteria are called ligands and those on host cells, receptors. The interaction of the bacterial ligands with host cell receptors can be compared to the well-known antigen–antibody or plant lectin–sugar interactions (Sharon and Lis, 1972). Thus, the specificity of the interaction can be demonstrated by

(1) inhibiting the interaction with large excesses of 'haptens' either identical to or resembling the native ligand or the native receptor (Fig. 1.3),
(2) chemical or enzymatic treatment of the bacteria or tissue cells to abolish or alter the specific surface structures involved in adherence, and
(3) blocking the ligand or receptor with specific antibodies directed against antigens composing these structures.

The demonstration of the ability of bacterial or host cell surfaces to bind the