

HOST-MICROBE RELATIONSHIPS

By W. J. Nungester

Department of Bacteriology, University of Michigan, Ann Arbor, Mich.

Host resistance is one of the two major factors controlling the infectious process, pathogenicity of the infecting organism being the other. This has been recognized since the early days of microbiology. It is recognized that certain general factors play important roles in host resistance. The age of the individual, his nutrition, the general state of his health, and other general factors all must be considered in defining host resistance. Also, the experience of the host with respect to previous exposures to antigens as a result of clinical or subclinical disease, or active immunization, may contribute materially to specific resistance or immunity. In this presentation, however, attention will be directed primarily to natural or innate host resistance.

Progress has been made in resolving the general factors of natural or innate host resistance into specific mechanisms. Our knowledge of the humoral factors, particularly the action of blood serum on microorganisms, has increased somewhat since it was first observed that heat-labile factors of serum acted on gram-negative bacteria, and that the heat-stable factors, the beta lysins, possessed antimicrobial properties against the gram-positive microorganisms.

The heat-labile or complement factors in blood serum have been extensively studied, particularly as they affect the hemolytic reactions.^{1, 2} We are not too certain, however, as to the relationship of hemolytic complement and the heat-labile antimicrobial factors in serum, sometimes referred to as bactericidal complement. Possibly bactericidal complement requires a fifth component for its action. The discovery of properdin by Pillemer³ may help in resolving the apparent difference between hemolytic and bactericidal complement.

Since there is currently a strong tendency to relate certain strains of *Escherichia coli* to infectious diarrhea of the newborn, it would seem appropriate to determine the action of the bactericidal serum complement components, including properdin, on the particular strains of *E. coli* thought to be the cause of this disease. If bactericidal complement does act on these strains, which may or may not be true, then the question arises as to whether these antimicrobial factors are present at birth.

Dancis and Kunz⁴ have noted that the bacteriostatic activity of serum of both full-term and premature infants at birth was not too different from that of older children or adults, but that this activity tended to disappear within a few weeks and then to reappear after a year or so. This lack of bactericidal action of serum of infants older than two weeks against gram-negative organisms (*E. coli*, *Salmonella derby*, and *Neisseria catarrhalis*) was not related to hemolytic complement but appeared to be associated with a factor in serum gamma globulin.

Other body fluids, such as gastric, pancreatic, and intestinal secretions, may well be concerned in the resistance of the infant to the entrance of microorganisms by way of the gastrointestinal tract. Unfortunately, very little has been

done to evaluate the role of such secretions in the host resistance of man at any age, let alone that of the infant.

It is currently recognized that bacterial ecology may play a role in host resistance. Disturbances in the bacterial flora of the intestinal tract produced by the administration of antibiotics may result in an overgrowth of potential pathogens as *Micrococcus pyogenes* or the yeasts usually present in small numbers in this area. Miller and his colleagues⁵ have recently reported a tremendous decrease in the resistance of mice to *Salmonella* following a disturbance of the normal flora of the mice by preinoculation treatment with streptomycin. In recent studies in our laboratory, James Crawford has investigated the antagonism of bacteria isolated from the upper respiratory tract of man.⁶ He found that of 33 strains of gram-positive cocci isolated from patients, 26 inhibited pneumococcus type I, 4 inhibited group A *Streptococcus* type 3, and 4 were able to prevent growth of *N. catarrhalis*. Other groups of bacteria from patients, such as gram-positive and gram-negative rods and *Neisseria*, also had some bacteriostatic effect on the test organisms investigated. If the normal flora does act as a defense mechanism, obviously the infant may be vulnerable to certain organisms that normally are held in check in the older host by the established flora.

Microorganisms in the inhaled air impinge on the mucus layer of the respiratory tract. They are either destroyed by the defense mechanisms in this tract or are moved by ciliary action to the oral pharyngeal area and swallowed. Thus airborne organisms, including the etiological agent of infant diarrhea present in hospitals, will reach the gastrointestinal tract unless they are destroyed in the upper respiratory tract. Bacterial ecology of the upper respiratory tract, as well as of the intestinal tract, therefore, must be considered in a study of host resistance to the causative agent of diarrhea of the newborn.

The phagocytic cells of the host are recognized as playing an important role in host resistance. There is general agreement as to the various types of cells that possess phagocytic properties. These, of course, include the circulating and fixed phagocytes. The first prerequisite for phagocytosis is contact between the phagocyte and the microorganism. Chemotaxis, or attraction of wandering phagocytic cells to bacteria, may be either positive or negative in sense, depending on the microorganism concerned. The physical structure of the medium in which the phagocytic cells and bacteria are found may be quite critical in determining whether or not phagocytosis occurs. Wood and his colleagues⁷ have emphasized this phase of the problem under the title of "surface phagocytosis." The fixed phagocytes of the spleen, liver, bone marrow, *etc.*, may be aided in making contact with bacteria carried to them by the blood stream by virtue of a decreased rate of flow of blood through a large capillary bed. Also, it is possible that blood flow may actually be momentarily stopped in certain capillaries in the bed by a valvular control of these capillaries as suggested by Knisely.⁸ If we admit the possibility of such a valvular mechanism, the question then arises, at what age in the developing infant does it begin to function?

Once contact between the pseudopod of the phagocyte and microorganism is made, phagocytosis may or may not occur, depending on the presence in the

serum of normal opsonins, immune opsonins, or the nature of the phagocyte, the strain of the microorganism, and other factors such as an adequate ascorbic acid level in the phagocytic cell.^{9, 10} Something, but not enough, is known of the comparative physical and chemical properties of the phagocyte and the microbe as affecting this important defense mechanism. It is confusing to realize that both phagocyte and microorganism carry an over-all negative charge.¹¹ Possibly if we knew more of the dynamics of this process, this anomaly might be resolved by finding that the portion of the pseudopod, or at least its advancing portion making contact with the microorganism, actually carried a positive electrostatic surface charge.

It has often been stated that the bacterial capsule interferes with phagocytosis, which may or may not be true, depending on the host of origin of the phagocyte and the strain of pathogen. The chemical nature of the capsule, as a factor determining whether or not phagocytosis will occur, appears to be more important than its physical structure.

Once phagocytosis has occurred, the pathogen must be destroyed if this process is to be an effective host-defense mechanism. For many years it has been realized that phagocytic cells varied markedly in their ability to destroy microorganisms. Whether or not the microbe is or is not destroyed depends on such factors as the host of origin of the phagocyte, the type of phagocytic cell, whether or not the host has been previously immunized, and the strain of microbe. The enzymatic mechanisms by which microbes are destroyed within phagocytic cells have not been clearly defined to date. Advances are being made, however, in the general enzymology of phagocytic cells. Various enzymes have been isolated from these cells,¹² their metabolisms have been studied to a limited degree,¹³ and a few investigations on the effect of fractions of the pathogens on the metabolic mechanisms of leukocytes from resistant and susceptible hosts have been made. In our laboratory, some comparative studies of this nature have been done by Alonso,¹⁴ who has found that the specific polysaccharide of type I pneumococcus stimulated anaerobic acid production by rat leukocytes about 10 times more than it stimulated the same metabolic activity of guinea pig leukocytes over a period of observation of 90 minutes. The guinea pig is many times more resistant to the pneumococcus type I than is the rat.

Since energy is required for all phases of the phagocytic process, Murayama,¹⁵ in our laboratory, studied the effect of pneumococcus type I polysaccharide and the anthrax-immunizing antigen of Wright¹⁶ on the adenosine triphosphatase (ATP'ase) activity of this enzyme isolated from rat and guinea pig leukocytes. These animals vary markedly and in an opposite sense in their susceptibility to the pneumococcus and *Bacillus anthracis*. Muryama found that the ATP'ase from the leukocytes had greater activity in the presence of these "virulence factors" if the enzyme came from a resistant animal, and was depressed if the enzyme source was a susceptible animal.

Since the ultimate conflict of the pathogen and the host is decided at a biochemical level, it would seem very desirable that more comparative biochemical investigations be made of host resistance mechanisms.

Bacon, Burrows, and Yates¹⁷ have found that certain strains of *Salmonella*

unable to synthesize para-aminobenzoic acid (PAB) would not produce infection in mice unless the level of available PAB in the mouse was artificially raised. This approach to a biochemical basis for host resistance emphasizes the possibility that host resistance may depend on the absence of a necessary growth factor for the parasite. Conversely, of course, the presence of an antimicrobial factor in the host may prevent growth of the parasite. Obviously, both approaches must be developed if an adequate understanding of host resistance is to be acquired.

The mechanism of disease production is an important consideration in natural host resistance. Unfortunately, very little is known of the actual mechanisms involved in the production of signs and symptoms of disease in most infectious diseases. Certainly, infant diarrhea is one such disease in which our knowledge of the pathogenesis is lacking. The difference in susceptibility of two infants equally exposed to the etiological agent of infant diarrhea may depend in part on the ability of the organism to survive and grow in the body and in part to the reaction of the infant to the biochemical factors elaborated by microbial growth.

Although there is little definitive knowledge as to how microorganisms produce disease, it is in order to review briefly what information we do have.

In some infections there is actual destruction of vital tissues of the body, as in poliomyelitis, syphilitic aortitis, and hepatitis. How these tissues are destroyed is not obvious, yet the fact that we know that they are gives some satisfaction in explaining the pathogenesis of these diseases.

In botulism it is now known that the toxin blocks the transmission of the nerve impulse across the neuromuscular junction. This gives an adequate explanation of the cardinal signs of this disease.

As a general proposition it may be said that the circulating blood is altered in an infectious disease. The changes that occur may impose an increased burden on the cardiovascular system, as seen in the intravascular clumping of red blood cells by bacterial polysaccharides which Youngner and Nungester observed in 1944¹⁸ or in the related "sludging" of blood as reported by Kniseley.¹⁹ The latter phenomenon is by no means limited to infectious disease. In either case a microscopic observation in arterioles of the circulating blood so affected impresses one with the increased cardiovascular effort required to keep the affected blood moving at an adequate rate.

The recent publication of Smith and his colleagues from England²⁰ on the pathogenesis of anthrax directs our attention to the effect of microbial infections on such subtle physiological reactions as shock, with its endocrinological implications. The introduction of ACTH and related compounds into medical practice has not only made possible the control of some of the signs and symptoms of the infectious process, but has suggested that disturbance of the patient's endocrine physiology may be an important factor in disease production in infections.

Miller²¹ has reported that the endotoxin of the meningococcus interferes with glycogenesis by the liver. The current interest in electrolyte distribution between tissues and body fluids in shock and other clinical conditions may ultimately lead to a better understanding of the origin of signs and symptoms

in infectious diseases. Certainly in infant diarrhea there is marked alteration in electrolyte balance as a result of the infectious process.

In summary, it must be assumed that host resistance to a given parasite is an algebraic summation of the various defense mechanisms of the host. In the future it may be possible to set up an equation with appropriate qualifying exponents to describe the total resistance of the host. This is currently done to a limited degree in describing the resistance of the immunized host with respect to circulating antibodies. Even here, this approach is not entirely adequate, since no measure is made of intracellular antibodies and since the natural or innate defense mechanisms are not adequately considered as an important component of the entire picture.

Biologists, as a class, are not sympathetic to the mathematical approach. Two reasons for this are apparent. First, much of our data is not quantitative and, second, we, as biologists, usually lack a mathematical background. As our sciences mature, both defects will be corrected. Hence, we can look forward to a more exact description of host defense mechanisms even on a mathematical basis.

References

1. PILLEMER, L., E. E. ECKER, J. L. ONCLEY & E. J. COHN. 1941. Preparation and physicochemical characterization of serum protein components of complement. *J. Exptl. Med.* **74**: 297-308.
2. MOYER, M. M. & L. LEVINE. 1954. Kinetic studies on immune hemolysis. IV. Rate determination of the Mg^{++} and terminal reaction steps. *J. Immunol.* **72**: 516-530.
3. PILLEMER, L., L. BLUM, I. H. LEPOW, O. A. ROSS, E. W. TODD & A. C. WARDLAW. 1954. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science*. **120**: 279-285.
4. DANCIS, J. & H. W. KUNZ. 1954. Studies of the immunology of the newborn infant. VI. Bacteriostatic and complement activity of the serum. *Pediatrics*. **13**: 339-345.
5. MILLER, C. P., M. BOHNHOFF & B. L. DRAKE. 1954. The effect of antibiotic therapy on susceptibility to an experimental enteric infection. *Trans. Assoc. Am. Physiol.* **67**: 156-161.
6. CRAWFORD, J. G. 1955. Antibacterial properties of human upper respiratory secretions. Doctoral Thesis. Univ. Michigan. Ann Arbor, Mich.
7. WOOD, B. W., JR., M. R. SMITH, W. D. PERRY & J. W. BERRY. 1951. Studies on the cellular immunology of acute bacteremia. I. Intravascular leucocytic reaction and surface phagocytosis. *J. Exptl. Med.* **94**: 521-533.
8. KNISELY, M. H. 1940. The histophysiology of peripheral vascular beds. Blood heart and circulation. *Am. Assoc. Advance. Sci.* **13**: 303.
9. COTTINGHAM, E. & C. A. MILLS. 1943. Influence of environmental temperature and vitamin deficiency upon phagocytic functions. *J. Immunol.* **47**: 493-502.
10. NUNGESTER, W. J. & A. M. AMES. 1948. The relationship between ascorbic acid and phagocytic activity. *J. Infectious Diseases*. **83**: 50-54.
11. NUNGESTER, W. J., A. M. AMES & W. LANNING. 1952. Electrophoresis studies of leucocytes and bacteria in relation to the mechanisms of phagocytosis. *J. Infectious Diseases*. **90**: 61-66.
12. REBUCK, J. 1947. Functions of white blood cells. *Am. J. Clin. Pathol.* **17**: 614-630.
13. VALENTINE, W. N. 1951. Quantitative biochemical studies on leukocytes in man: a review. *Blood*. **6**: 845-854.
14. ALONSO, D. 1952. A comparative study of the metabolism of leucocytes from guinea pigs and rats. Doctoral Thesis. Univ. Michigan. Ann Arbor, Mich.
15. MURAYAMA, M. 1955. Comparative study of host resistance of guinea pigs and rats. IV. The effect of bacterial fractions on the adenosinetriphosphatase activity of the guinea pig and rat leucocyte nuclei-free homogenates. *J. Infectious Diseases*. **97**: 1-7.
16. WRIGHT, G. G., M. A. HEDBERG & R. J. FEINBERG. 1951. Studies on immunity in anthrax II *in vitro* elaboration of protective antigen by nonproteolytic mutants of *Bacillus anthracis*. *J. Exptl. Med.* **93**: 523-527.

17. BACON, C. A., T. W. BURROWS & M. YATES. 1951. Effects of biochemical mutation on the virulence of *Bacterium typhosum*. Brit. J. Exptl. Pathol. **32**: 85-96.
18. YOUNGNER, J. S. & W. J. NUNGESTER. 1944. The effect of type III pneumococcus polysaccharide and gelatin on the circulation and sedimentation of erythrocytes in mice. J. Infectious Diseases. **74**: 247-253.
19. KNISELY, M. H., T. STRATMAN & T. S. ELIOT. 1941. Capillary circulation in the malaria infected monkey. A cinematographic study. J. Am. Med. Assoc. **116**: 2430-2431.
20. SMITH, H., J. KEPPIE & J. L. STANLEY. 1955. The chemical basis of the virulence of *Bacillus anthracis*. V. The specific toxin produced by *B. anthracis in vivo*. Brit. J. Exptl. Pathol. **36**: 460-472.
21. MILLER, C. P. 1933. Experimental meningococcal infection in mice. Science. **78**: 340-341.