

SECTION IV. HOST-PARASITE INTERACTIONS: HOST DETERMINANTS

SPECULATIONS ON THE IMMUNOLOGY OF STAPHYLOCOCCAL INFECTIONS*

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Eight years ago increasing problems with human staphylococcal disease led to a New York Academy of Science conference on staphylococcal infections.1 In comparing the papers presented at that time and the titles submitted for the present conference,2 it is encouraging to note that a considerable amount of new information has accumulated during this interval. More is known about the epidemiology of staphylococcal disease, the ways in which newborns acquire initial infection, and the circumstances in which staphylococcal infections arise in adults. New biochemical and ultramicroscopic information on the structure of the staphylococcal cell wall has emerged. Certain of the biochemical components which may play a role in virulence have been characterized; there is additional information on some of the peculiarities of staphylococcalhost cell interactions. The development of new antimicrobial agents, particularly the penicillinase-resistant penicillins, has altered and simplified therapy, and the prognosis in life threatening staphylococcal disease has been improved. Even location of the conference itself suggests increasing sophistication. The 1956 conference was held in the basement of another hotel which will remain nameless. The 1964 conference was held in the Waldorf Astoria. I hope that this is additional evidence of progress!

There is, however, one aspect of staphylococcal disease which has remained confusing and poorly understood. This is the problem of the nature of immunity or acquired resistance to staphylococcal infections in man. This lack of understanding does not stem from a paucity of experimental studies in this area—the literature dealing with various aspects of staphylococcal immunity is enormous. With certain outstanding exceptions, however, these studies have not clarified the broad problem of human resistance to staphylococcal infection. Most studies have dealt with isolated facets of the immune process, and the over-all problem has received scant attention. Like the blind men in Tolstoi's fable, conclusions have often been drawn from narrow evidence based on immunologic dogma acquired from study of other infections quite different from those produced by staphylococci.

Review of these studies in preparation for this conference has strengthened

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a belief first put forth in tentative fashion in 1961.3 I would submit that we have on hand considerable evidence that most humans develop impressive "immunity" to staphylococcal disease and that our problem is conceptual, stemming not from lack of evidence of immunity, but because there is too much of it. I shall thus not review all of the studies on the immunology of staphylococcal disease which have recently been summarized by Elek.4 Rather, I should like to propose a series of theses regarding staphylococcal immunity, and to select certain clinical and experimental evidence to support them. While much of what I shall have to propose is speculative, I believe these theses can be submitted to appropriate experimental study for proof or rejection.

The theses run as follows:

- (1) Normal adult humans possess a high degree of resistance to staphylococcal disease.
- (2) Much of this resistance is acquired through experience with staphylococci and can be explained on an immunologic basis.
- (3) Certain clinical features of staphylococcal disease are a resultant
- (4) The typical staphylococcal lesion, the abscess, may of itself alter of this immunity. immune response to staphylococcal infection.
- (5) Attempts to control problems of staphylococcal disease in man by evoking or reinforcing specific immunologic resistance are theoretically unsound and offer little hope of success.

What Staphylococcal Infections Are Not

In order to develop my argument, it is necessary to outline what staphylococcal infections are not. In my view, erroneous conclusions, have often resulted from attempts to equate staphylococcal infections with certain other infectious disease processes which differ in basic hostparasite interactions. Our current knowledge of staphylococcal disease permits reexamination of certain of these assumptions.

First, it is increasingly evident that staphylococci cannot be equated with microbes which produce human illness by the elaboration of extracellular products with biologic activity. The discovery of staphylococcal alpha toxin and its apparent role in the deaths which followed injections of contaminated diphtheria toxoid in Bundenberg, Australia, in the 1920's led early investigators to believe that staphylococcal infections could be approached in the same manner as diphtheria or tetanus. This is clearly not the case. There is abundant evidence that the dissemination and multiplication of living staphylococci at sites of infection is one of the hallmarks of staphylococcal disese. While controversy still exists, most evidence suggests that, while alpha toxin may play a role in the clinical syndrome observed following establishment of infection, 5.8.9 it cannot explain the initiation of the staphylococcal disease.

In a similar vein, I believe there is now good evidence to indicate that staphylococcal infection cannot be equated in any simple manner with infections caused by microorganisms which produce disease by multiplication exclusively at extracellular loci. While certain strains of staphylococci which possess a capsular structure are significantly more virulent in experimental animals by virtue of their resistance to phagocytosis, ¹⁰ 12 as yet there is no convincing evidence that all stains producing disease in man behave in this manner. ¹³ It is thus not surprising that attempts to equate staphylococcal infections with infections produced by microorganisms like the pneumococcus or Friedlander bacillus have similarly met with disappointment.

Lastly, it should be stated that, while staphylococcal disease is not a simple extracellular infection, neither is it a disease characterized by primary intracellular sites of residence of the microorganism. Although there are many analogies between infections produced by staphylococci and the tubercle bacillus, and intracellular survival may be an important biologic attribute of pathogenic strains, extracellular multiplication clearly plays an important role in clinical staphylococcal disease.

The Nature of Staphylococcal Infections

To render understandable the immunologic phenomena which have been observed, it is important to consider the features which do characterize the host-parasite relationship in staphylococcal infection. I believe there is much to indicate that staphylococcal infections have biologic facets of all three kinds of host-parasite interactions already described. Philosophically, this belief seems ecologically reasonable. Staphylococci and man have existed together for many centuries. Their ubiquity and their adaptability, both so evident during the antimicrobial era, makes it probable that staphylococci have multiple biological characteristics which contribute to their continuing symbiotic existence with human civilization.

That staphylococci produce potent extracellular toxins is well established. It would seem probable that they play a role in human disease. Certain symptoms seen in staphylococcal bacteremia can be mimicked by injections of alpha toxin in experimental animals. It seems likely that the leukocyte destroying factors recently reexamined by Gladstone, Mudd, and their associates, may play a role in aiding establishment of infection. It has been shown that coagulase can protect coagulase-positive staphylococci from factors in serum which inhibit their growth. Clearly, staphylococcal enterotoxin is an important cause of food poisoning—a situation where multiplication of microorganisms or their entry into tissues is not required. Despite this evidence, all would agree that elaboration of extracellular factors is not the whole picture. Staphylococci must multiply to high titers before elaboration of such products assumes im-

portant quantitative significance. It is thus understandable that humoral immunity directed at such extracellular factors often fails to prevent the establishment of staphylococcal infection.

That resistance to phagocytosis conferred by a capsular or surface polysaccharide can contribute to the virulence of certain strains of staphylococci has been clearly established. 10,11 Certain strains can behave very much like pneumococci in this regard, and immunization with this capsular substance can confer impressive protection in experimental systems.19 21 It should be pointed out, however, that such demonstrations require the use of an experimental animal which lacks detectable opsonin against such strains. Studies in human serologic systems indicate that most normal adults already possess such opsonins. 13,22,28 Further, immunity based on opsonins which promote phagocytosis requires that the phagocytic process result in rapid killing of the ingested parasite. When this occurs (for example, in pneumococcal infections), antibody directed at capsular antigen confers impressive immunity. In dealing with an organism like the staphylococcus which may survive within cells, it seems unlikely that opsonins can confer the same clear-cut resistance to infection.

This leads me to the last characteristic of staphylococcal-human host interactions. It is now well established that coagulase-positive staphylococci capable of producing disease in man possess the ability to survive within the interior of polymorphonuclear leukocytes and perhaps within monocytes as well.²⁴ ²⁸ While phagocytosis is a vital feature of host defense and clearly destroys many staphylococci, the ability of a small number of microbes to survive long periods of residence within leukocytes may have important biologic significance.

Experimental evidence supports the thesis that the host-parasite interactions in staphylococcal infection is multifactorial. Using a carefully defined mouse model, studies by Cohn²⁹ and Koenig^{19,11,21} have shown the following:

- (1) That the relationships of the number of viable staphylococci to the number of available phagocytic cells in the peritoneum are critical. Minor variations in this ratio can profoundly change the outcome of this interaction, irrespective of the immune mechanisms under study. The addition of mucin to the inoculum can further alter these interrelationships.³⁰
- (2) That the resistance of the Smith diffuse strain to phagocytosis is critical in the initiation of infection. Opsonin can modify this initial interaction profoundly if proper staphylococcal to phagocytic cell ratios are maintained.
- (3) That after peritoneal infection is established, alpha hemolysin probably plays an important role in the outcome. When progressive infection is underway, increasing amounts of alpha toxin are produced, eventually killing mice even though they possess high antitoxin titers.

Thus, in one simple experimental model one can demonstrate that the ratio of organisms to available phagocytic cells, the presence or absence of antibody against opsonin, and the elaboration of alpha hemolysin in vivo can all play a role in the outcome. Clearly, conclusions derived from attention directed toward any single one of these factors would be in error.

Thus, it does not seem surprising that the immunology of staphylococcal disease appears puzzling. Antibody promoting phagocytosis cannot be fully protective. By the same token, antibodies directed toward extracellular products elaborated by staphylococci cannot produce clear-cut results. The immunology of infections characterized by intracellular loci of residence has been notoriously difficult to unravel. What then is the evidence that humans do possess or acquire immunity to staphylococcal disease?

Clinical Evidences of Acquired Immunity

If one assesses the spectrum of clinical staphylococcal infections as one moves from birth to adulthood, one can develop considerable indirect evidence to show that humans acquire resistance to staphylococcal disease. The newborn infant is highly susceptible to infection by staphylococci. The majority of infants are colonized with potential disease producing strains within the first four to five days of life. While this may be explained by the presence of a raw umbilical stump, or differences in newborn skin, it is equally reasonable to suppose that this susceptibility derives from relative absence of immunity in early infancy. Further, the nature of the staphylococcal lesions as they occur in the infant are distinctly different from staphylococcal lesions observed in the adult. There is a greater tendency for these infections to be diffuse and poorly focalized,31 and there is some evidence to suggest a considerably higher incidence of blood stream invasion in children with staphylococcal infections.31.32

In the adult, the frequency of staphylococcal infection, as evidenced by studies on the carrier state in contrast to the rarity of invasive disease, is of itself suggestive of immunity. The fact that most life threatening infections in adults occur in concert with diseases or exhibition of drugs known to alter or reduce resistance gives further support to the thesis that immunity plays an important role in prevention of staphylococcal disease in normal humans. The huge numbers of staphylococci required to produce detectable infection in the adult can be cited as further evidence of immunity. While 50 to 100 Treponema or Pasturella tularensis will produce experimental infection in man, over a million staphylococci must be used to establish cutaneous infection in man.33

Lastly, the very nature of the staphylococcal lesion, the abscess, suggests previous immunologic experience. A striking tendency to focalize with a prompt and intense cellular reaction are characteristic of the immune process. Studies by Johnson, Cluff, and Goshi, indicating that hypersensitivity may play a role in the nature of the lesion,34 further support this thesis. Thus, clinical evidence of immunity, while indirect, is highly

To turn briefly to another aspect of the staphylococcal abscess, it is worth considering that the abscess lesion may itself alter immunologic response. The containment of staphylococci within a fibrinous or fibrous capsule may prevent antigens from escaping to reach antibody forming centers. Hughes has shown that fibrin membranes may act as a selective filter.35 Experimental studies by Hite, Banks, and Dack,36 and unpublished

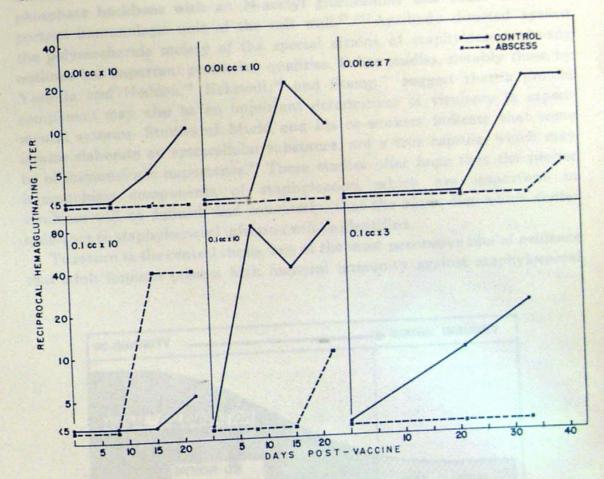
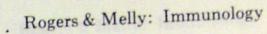


FIGURE 1. Effect of the abscess on antibody production.

studies from our own laboratory suggest that antibody response may be blunted by containment of antigen within a cavity. FIGURE 1 shows the differences in antibody response obtained in six pairs of rabbits injected with a staphylococcal antigen. One member of each pair received antigen introduced into a sterile abscess-like cavity, while the control animal received antigen subcutaneously. Thus, the abscess may serve to isolate antigen and prevent further immunization of the host.



DETECTABLE ANTIBODY LEVEL

FIGURE'2. Typical antibody response to repeated antigenic stimuli.

Laboratory Evidences of Acquired Humoral Immunity

There is abundant evidence that most adult humans possess an impressive array of antistaphylococcal antibodies.3 As a generality, virtually all substances derived from staphylococci which have been found to be antigenic have been found to have their antibody counterparts in most adult human sera. Numerous studies with whole staphylococcal cells have shown that most humans possess antistaphylococcal bacterial cell agglutinins.3 Recent studies by Jensen showed that 100 per cent of 500 adult sera had demonstrable agglutinins against a polysaccharide fraction.38 Similar evidence has been obtained with many of the extracellular products of staphylococci. Most adults possess demonstrable levels of alpha antitoxin3,38 and antileukocidin.39 Rammelkamp has shown that children possess lower titers of anticoagulase than do adults, but again, virtually all adult sera showed the presence of antibody.31 The clumping of coagulasepositive strains of staphylococci noted when they are grown in soft agar containing human serum or plasma appears to be an antigen-antibody reaction.3 Again, virtually all normal adult plasmas possess this capacity. Our own studies have shown that virtually all adults have staphylococcal hemagglutinins, a relatively nonspecific measure of antistaphylococcal antibody.13 Humans possess opsonins which not only promote the phagocytosis of most wild type strains, but also the special strains of incapsulated staphylococci studied by a number of investigators. 13,20,22

One of the problems which has made interpretation of immunologic events confusing has been the lack of fundamental knowledge regarding the antigenic structure of staphylococci. This is now being corrected. Within the last several years, a number of studies on the biochemistry of the staphylococcal cell wall have been carried out which promise to give us better defined antigens and more definitive information on the nature and importance of antibodies directed against these substances.40 47 Current evidence indicates that both polysaccharide and protein components of the staphylococcal cell may be immunologically significant. Studies by a series of investigators indicate that a teichoic acid formed of a ribitol phosphate backbone with an N-acetyl glucosamine side chain is an important immunologic unit of the cell wall.40-44 Antibody directed against the polysaccharide moiety of the special strains of staphylococci already outlined has important protective qualities. Other studies, notably those by Yoshida and Hedden, 45 Eckstedt, 46 and Stamp, 47 suggest that a protein component may also be an important determinant of virulence in experimental systems. Studies of Mudd and his co-workers indicate that some strains elaborate an extracellular substance, not a true capsule, which may be of immunologic importance.48 These studies offer hope that the precise immunologic components of staphylococci which are important in virulence will be defined, and conversely, that the antibodies which confer resistance to staphylococcal infection will be identified.

To return to the central thesis, one of the most persuasive bits of evidence that adult humans possess high humoral immunity against staphylococcal

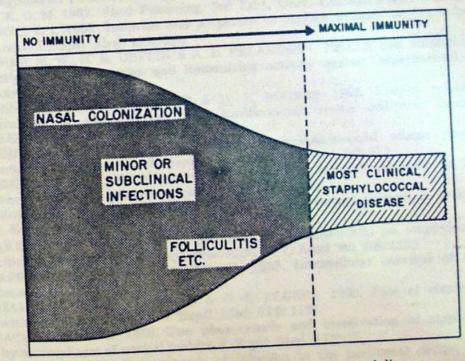


FIGURE 3. Immunologic status in staphylococcal disease.

Rogers & Melly: Immunology

infections can be derived from observations on the immunologic status of humans with overt staphylococcal disease. In the main, studies on patients with active staphylococcal infection have shown that these individuals do not demonstrate higher titers of a variety of antistaphylococcal antibodies than do normal adults. 3,32,36,38,45 Further, several studies indicate that immunization of humans with staphylococcal toxoids or vaccines of proven antigenicity in animals often fails to change antibody

I believe the most reasonable explanation for these observations lies in titers in man. 49 the status of immunity in apparently normal humans. This thesis is diagrammed in FIGURE 2. As shown here, nonimmune humans and experimental animals respond in predictable fashion to the administration of antigens. Initial injections produce a small antibody response. Subsequent injections evoke swifter and more magnified antibody production. Eventually, however, one arrives at a state in which further antigen does not evoke further increase in antibody production. In all probability, antibody synthesis is proceeding at maximal rates and cannot be further reinforced. I believe that most adult humans have arrived at this state. The nasal carrier state, coupled with repeated experiences with subclinical or minor infections and furuncles, may operate to maintain humoral antibodies at maximal titers.

I thus believe that most clinical staphylococcal disease as we observe it in humans represents infection superimposed on a high degree of humoral immunity (FIGURE 3). Such a concept makes the immunologic peculiarities of staphylococcal disease understandable. That disease can emerge despite a high degree of immunity may be due to inoculum size, disease processes, or manipulations which reduce resistance to infection; or because of other unknown but nonimmunologic reasons.

If this thesis is correct, it seems unlikely that immunologic approaches to staphylococcal infection can offer many solutions to staphylococcal disease. To borrow a phrase from the musical "Oklahoma," I would guess that human antibody responses to staphylococci "have gone about as fur as they kin go." Other approaches—that of selecting the kind of staphylococcus we allow to colonize man as explored by Schinefeld and his associates, 50,51 or developing ways to reinforce nonimmunologic resistance to staphylococcal infection-seem to me more profitable avenues for exploration.

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THE PHAGOCYTOSIS AND INTRACELLULAR FATE OF STAPHYLOCOCCI

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The problem of studying the phagocytosis and intracellular fate of Staphylococcus aureus has received attention from several investigators during the last decade.1-9 Although discrepancies in conclusions were evident initially, subsequent studies have, for the most part, brought about resolution of these difficulties. It is probably safe to say the general concensus is, that once phagocytized, Staph. aureus is usually subject to killing by both neutrophils and mononuclear cells, but with certain exceptions to be pointed out later. No evidence has been presented to indicate that this organism is capable of continuous multiplication within phagocytes. It appears that opsonins of some type are probably necessary in order to achieve phagocytosis. This is particularly evident in studies employing the Smith encapsulated strain where little or no phagocytosis takes place unless specific anticapsular antibodies are present in the system.3 This requirement for opsonins is more difficult to show with conventional strains, presumably because of the widespread occurrence of antibodies against staphylococci in the mammalian species usually employed in such studies. Studies of Mudd et al.10 indicate that the specificity of opsonins for nonencapsulated strains may be directed toward the teichoic acid moiety of the cell wall rather than the common protein agglutinogen.

It is also apparent that certain heat-labile components, presumably complement or complement-like material, aid in phagocytosis and are necessary for the subsequent intracellular destruction.11,12 The studies of Hirsch and co-workers have offered a reasonable explanation for the actual mechanism of killing within neutrophils, whereby the lysosomal contents are discharged into the phagocytic vacuole.13 Once this occurs a considerable degredation of bacterial constituents begins. 14,15

A factor not to be overlooked in studies of this nature is methodology. No one method is applicable to all types of studies relating to these problems and on some occasions do not necessarily come up with the same answers. The data presented here were obtained by using either a modification of the method described by Malløe or the tissue-culture chamber procedure of Kapral and Shayegani.

I should, first of all, like to discuss the interaction of Staph. aureus with neutrophils. In the normal adult human, neutrophils derived from the blood are readily capable of phagocytizing Staph. aureus in the presence of fresh serum and subsequently killing the majority of organisms within a few hours (FIGURE 1). Aliquots of the same neutrophil preparation, when mixed with Staph, aureus in the presence of heat-inactivated serum from the same