Host determinants of staphylococcal infections

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INTRODUCTION

It is a great privilege for me to be a participant in this centenary celebration of Alexander Ogston's work on the staphylococcus. My beginning work in the laboratory on the biology of staphylococci was inspired and stimulated by the observations of Professor Ogston although indirectly through the book by Stephen D. Elek, Staphylococcus pyogenes *and its Relation to Disease,* published in 1959 by E. and S. Livingston. This excellent book came into my possession during my first months in the laboratory when I struggled as a clinically trained pediatrician to learn the discipline of microbiology.

I was greatly impressed by the observation of Professor Ogston. He was a clinician as well as investigator and above all had a concern for patients. Ogston considered the role of host factors as well as the virulence of microorganisms in his attempts to explain human disease. An example is the following quote from Ogston's report in 1882, '. . . understanding of the relations between bacteria and the system cannot be attained until we have arrived at a definite knowledge concerning the peculiar influence of the living tissues that are so hostile to the life of microorganisms' (Ogston, 1882). Ogston must have been delighted when the role of phagocytes and opsonins were identified as important host factors in resistance to staphylococcal infections.

My talk is listed as the Davidson Lecture, and I am pleased to discuss pathogenic mechanisms of infectious disease, honouring this giant of Scottish medicine. Sir Stanley Davidson became Regius Professor of Medicine in Aberdeen in 1930, at the exact midway point between Ogston's

This paper includes the substance of the Davidson lecture delivered by Professor Quie.

original description of the staphylococcus and our celebration this year. Dr Davidson's textbook, *Principles and Practice of Medicine* is well-known throughout the world and his interest in infectious disease is reflected by a series of papers on Weil's disease among fish workers in Aberdeen. These papers were reported in the *British Medical Journal* in the 1930s and describe the disease in over 100 patients (Davidson & Smith, 1939). The University of Aberdeen has certainly been a rich source of knowledge in many aspects of infectious disease and it is appropriate that this centenary celebration is in the city of Aberdeen.

I am delighted to be able to share reports of some of the recent work from our laboratory at Minnesota with you and participate with other investigators at this symposium who are trying to understand more about how the staphylococcus usually lives in symbiosis with its hosts and what goes wrong when there is staphylococcal disease.

Host Defence

My own research began with a study of the effect of staphylococcal products on humoral factors in blood. The staphylococcus was reigning supreme as a pathogenic bacterium at this time since most strains in hospitals were resistant to penicillin and we were forced to learn more about the epidemiology of this organism and especially about host factors involved in pathogenesis and resistance. A clot is formed when staphylococci are incubated with human plasma through an interaction between staphylocoagulase and coagulase reacting factor which converts fibrinogen to fibrin. This was an old observation and I started working on staphylococci. There was interest in the fact that lysis of this clot eventually takes place. The mechanism of clot lysis was believed to be direct proteolysis. Dr Lewis Wannamaker observed a unique phenomenon produced by the staphylococcus; the Muller Phenomenon, i.e. punctate satellite areas of proteolysis around colonies of *S. aureus* when cultured on agar with a protein substrate such as haemoglobin and when there was serum in the growth medium. We studied the extracellular products of staphylococci and demonstrated that staphylococci produce staphylokinase which activates serum plasminogen so that it becomes an active proteolytic enzyme which digests haemoglobin in satellite areas around staphylococcal colonies (Quie & Wannamaker, 1969).

Plasmin is active against many host factors including complement proteins, suggesting that staphylokinase may be a significant virulence factor of *S. aureus*. Patients with staphylococcal disease develop antibodies

to staphylokinase (Quie & Wannamaker, 1964). There is little difference, however, between antibody levels in patients with staphylococcal disease and controls and, therefore, there is little evidence that this extracellular enzyme is a major determinant of staphylococcal pathogenicity. Staphylokinase is similar to other staphylococcal factors, including toxins, in the sense that antibodies to these extracellular bacterial factors cannot be correlated with protection against staphylococcal disease (Mudd, 1970).

Phagocytosis

Staphylococci are not killed by serum, therefore, phagocytosis by polymorphonuclear neutrophils or macrophages is necessary for host defence against staphylococcal disease. Our laboratory has concentrated on investigation of the staphylococci with phagocytic cells and serum factors which influence this interaction during the past two decades.

Protein A is a surface factor on nearly all strains of *S. aureus* which binds to immunoglobulins in serum without immune specificity. Protein A binds to the Fc part of the immunoglobulin molecule so that antibodies may not be effective as opsonin. Furthermore, protein A released from the organisms activates complement in the fluid phase and complement, therefore, is no longer available for attachment to the bacterial surface and serve its function as an opsonic amplifying mechanism (Dossett *et al.*, 1969). Evidence from our laboratory suggests that protein A may play a role in the severity of staphylococcal infection. For example, protein A rich strains are phagocytized poorly when serum has a high concentration of IgG and, in addition, there is reduced amplification of opsonic effect by complement. Protein A enhanced phagocytosis when immunoglobulins were deficient in serum which suggests that complement is directly activated on the surface of bacteria without immunologically specific antigen-antibody in the presence of protein A (Peterson *et. al.*, 1977).

The cell wall of *S. aureus* contains peptidoglycan (mucopeptide) which consists of peptide units connected to pentaglycine bridges and it is this structure which gives the cell wall its rigid structure. Staphylococcal cell walls also contain teichoic acids, either ribitol or glycerol and patients with prolonged staphylococcal disease develop high titres of antibody to teichoic acid which is useful as a serologic diagnostic tool (Crowder & White, 1972). Both cell wall peptidoglycan and teichoic acid are anti-phagocytic factors and staphylococci may also possess a capsular structure which is anti-phagocytic.

Staphylococcal opsonins

Opsonization is necessary for efficient phagocytosis of *S. aureus* (Wright & Douglas, 1904). Patients develop heat-stable serum opsonins after severe staphylococcal infections. For example, patients with staphylococcal endocarditis were found to have high titres of heat stable opsonic antibodies which belonged to the IgG class of immunoglobulins (Messner *et al.*, 1968). Although these patients had high titres of opsonic activity in heated serum there was equal opsonic activity in fresh serum from patients and from controls suggesting that complement is a primary opsonin for *S. aureus*.

When factors which block the Fc fragment of IgG, such as anti-gammaglobulin factors and protein A are present in serum or when antibody molecules are altered by pepsin or epsilon aminocaproic acid, opsonic activity is depressed. These findings suggest that opsonic antibodies, in addition to neutralizing anti-phagocytic factors on the surface of bacteria also act as ligands binding bacteria to the surface of phagocytic cells. The Fab part of antibody molecule attaches with immunological specificity to the surface of the bacteria and the Fc portion attaches to receptors on the phagocyte. The host determinants that are required for efficient opsonization and phagocytosis include antibodies both specific and cross-reacting and complement factors which may be activated by either classical or alternative pathways.

A method has been established in our laboratory using (^{3}H) thymidine labelled bacteria which allows simultaneous, independent evaluation of the process of opsonization, attachment, ingestion, and killing of staphylococci (Verhoef *et al.*, 1977). The bacteria are opsonized in serum from a variety of sources, i.e. with and without complement factors and with and without immunoglobulin. Uptake of the labelled bacteria by phagocytes was determined at several time points during incubation to determine kinetics of phagocytosis. Using this method with chelated serum or C_2 deficient serum as reagents allow measurements of the pathway of complement activation. *S. aureus* were slowly opsonized via the alternative pathway. Opsonization via the classical pathway was complete within 5 min while 60 min was necessary for opsonization via the alternative pathway. Once complement (presumably C3b) was attached to the bacterial surface, however, the organisms were efficiently phagocytized. Bacteria opsonized without complement were not only less efficiently attached to the phagoctye but more slowly engulfed. These findings confirm and provide the molecular basis for Dr Mudd's observation several years ago that staphylococci are more slowly engulfed by phagocytes in heated immune serum than in fresh normal serum (Mudd & Mudd, 1933).

The role of *S. aureus* cell wall peptidoglycan as an antiphagocytic factor neutralized by opsonization was extensively studied in our laboratory (Peterson *et al.*, 1978).

Peptidoglycan, a major component of the cell wall of *S. aureus* appears to be a scaffold to which peptides, lipids, and teichoic acids are attached. A variety of opsonins were used to determine opsonic requirements of these surface factors of staphylococci. Peptidoglycan in the absence of teichoic acid and teichoic acid-deficient mutants of *S. aureus* were opsonized and engulfed as rapidly as staphylococci with teichoic acid and with similar kinetics using several different opsonin sources which suggests that teichoic acid may not play a significant role in opsonization of *S. aureus* in normal serum. Its role in immune serum remains to be determined. *S. aureus* peptidoglycan was a potent activator of complement, especially via the classical pathway and there was a direct parallel between kinetics of complement activation and kinetics of opsonization. Staphylococcal cell wall peptidoglycan, therefore, by its property of associating with immunoglobulin and by activating complement plays a significant role in the recognition of staphylococci by phagocytic cells (Verhoef *et al.*, 1977).

Not all strains of staphylococci are opsonized and phagocytized efficiently by normal human serum because of a polysaccharide capsule (Peterson *et al.*, 1978). The capsular polysaccharide acts as a barrier to phagocytosis and specific anticapsular antibodies are necessary for engulfment of encapsulated staphylococci. We found that encapsulated variants of two strains of staphylococci (Smith and M) were poorly opsonized in a variety of serum sources when compared with non-encapsulated strains. Therefore, capsules appear to interfere with phagocytic cell recognition via both IgG receptors and C3b receptors. The only opsonin which brought about efficient uptake and killing of the encapsulated *S. aureus* variants was immune sera from animals immunized with encapsulated organisms. Encapsulated staphylococci were found to be capable of activating complement via both classical and alternative pathway and there was fixation of C3b on the encapsulated bacteria (Peterson *et al.*, 1977). Capsular material, therefore, does not interfere with complement activation by peptidoglycan or with association of the activated complement component C3b to the bacterial cell wall. It does somehow prevent recognition and, therefore, attachment to the phagocytic cell surface. Specific anticapsular antibodies neutralize the anti-phagocytic property of capsular antigens and are effective opsonins of encapsulated strains.

Intra-leukocyte killing of S. aureus

Phagocytes are essential for host defence against staphylococcal disease since this bacterial species is not susceptible to extra-leukocyte bactericidal mechanisms. Complement killing does not occur even in the presence of high titre antibody and staphylococci are not susceptible to lysozyme. However, once staphylococci are engulfed by phagocytic cells they are rapidly killed within phagocytic vacuoles. It is appropriate, therefore, that the mechanisms of intracellular killing of staphylococci should be considered. When the staphylococci or other particles are phagocytized by polymorphonuclear leukocytes there is a striking morphologic change which includes degranulation and phagocytic vacuole formation. An equally abrupt metabolic response results in oxygen metabolism and production of reactive oxygen radicals which, in turn, react with the ingested microbe within phagocytic vacuoles. The simultaneous release of granules into phagocytic vacuoles and respiratory metabolism results in activation of the microbicidal system within phagocytic vacuoles in juxtaposition with the sequestered microbes.

The myeloperoxidase, hydrogen peroxide, halide interaction is a potent antimicrobial system of human granulocytes (Klebanoff, 1975). Myeloperoxidase is present in high concentration in the azurophilic granules (lysosomes) of neutrophils and is released into phagocytic vacuoles during phagocytosis. Hydrogen peroxide and other oxygen radicals are generated by neutrophils. Hydrogen peroxide is also produced by certain catalase-negative species such as staphylococci but not by staphylococci which are catalase producers. Several halides are present in granulocytes such as chloride which plays a major role as a microbicidal co-factor since it is always present in high concentration and iodide which is available from thyroxine and triiodothyroxine during phagocytosis. The myeloperoxidase hydrogen peroxide reaction results in a complex with strong oxidizing activity and chloride or other halides are oxidized to labile reagents which react with bacterial cell walls and membranes. This reaction also results in generation of singlet oxygen which is highly reactive and emits energy as light termed chemiluminescence. This response correlates with the bactericidal capacity of granulocytes and is a useful test of granulocyte function.

Patients with Defective Host Defence

The importance of the oxidative metabolic burst for intracellular killing of staphylococci by granulocytes was identified when patients with great sus-

ceptibility to severe staphylococcal disease were found to lack the oxidative metabolic burst during phagocytosis. These patients had a normal immune response, normal numbers of granulocytes with normal morphology so their only defect was absence of the oxidative response during phagocytosis. This syndrome, termed 'Chronic Granulomatous Disease of Childhood' has served as a clinical model clearly demonstrating that clinical disease from *S. aureus* may be directly associated with phagocytic cell dysfunction (Quie, 1972).

Patients with severely defective phagocytic cell function such as chronic granulomatous disease suffer from staphylococcal lesions involving skin and subcutaneous tissue, lymph nodes, bones, lungs, liver, G-I tract, and G-U tract. Eczematoid skin lesions, suppurative lymphadenitis, and pulmonary lesions begin during early months of life and recur with discouraging frequency. Pulmonary lesions are frequently characterized by pneumatocele which may persist for several weeks in spite of intensive antimicrobial therapy. Staphylococcal osteomyelitis involving the small bones of the hands and feet occurs frequently and new lesions may appear during treatment. The lesions consist of purulent material with surrounding mononuclear cells which 'wall off' infected tissue. The macrophages which surround lesions often contain 'lipoid' material. The lesions caused by staphylococci in patients with granulomatous disease are quite similar histologically with lesions caused by *M. tuberculosis* in subjects with normal phagocyte function (Quie *et al.*, 1974).

The identification of granulocyte dysfunction as a cellular basis for staphylococcal susceptibility in chronic granulomatous disease stimulated research of other clinical conditions characterized by increased susceptibility to infection.

Several patients with defective leukotaxis and recurrent staphylococcal infections have been reported. Buckley and colleagues (1972) reported two adolescent males with IgE levels greater than 5000 ng/ml and recurrent severe abscesses due to *S. aureus*; Clark *et al.*, (1973) have described an 11-year-old female with similar findings. More recently, Hill & Quie (1974) reported three such patients with severe eczema early in life followed by recurrent, severe infections with extremely elevated levels of IgE and defective neutrophil chemotaxis. Hill *et al.* (1974) have also demonstrated defective neutrophil chemotaxis in several additional cases with recurrent cold staphylococcal abscesses and chronic eczema. The decreased leukotactic responsiveness of these patients was not related to a direct effect of the high concentrations of IgE, since the serum from these patients did not impair the chemotactic activity of control leukocytes. However, it has been demonstrated that there is elevated IgE anti-staphylococcal antibodies in

anti-staphylococcal antibodies in patients with this syndrome (Schopfer *et al.*, 1979). it is postulated that histamine and histamine-induced cyclic adenosine monophosphate may depress chemotactic responsiveness. These patients demonstrate that functional abnormalities of the immune response and phagocytic system are associated with increased susceptibility to staphylococcal disease. The formation of IgE antibodies to staphylococcal antigens may be a manifestation of altered antigen recognition or processing during infection with *S. aureus* which contributes to the chronicity or other clinical manifestations in patients with the hyper IgE syndrome. A study was done to determine if these IgE antibodies react with a specific component of the staphylococcal surface (Schopfer *et al.*, 1980). IgE antibodies were bound to cell walls of *S. aureus* but not other microbial species and binding was similar in teichoic acid-deficient mutants and in parent strains. These antibodies, therefore, appear to be directed against the cell wall peptidoglycan of *S. aureus*. Since recurrent severe infections with staphylococci characterize patients with the hyper-IgE syndrome, it is tempting to speculate that elevated IgE antibodies are an aberrant immune response to staphylococci and this abnormality may be related to increased susceptibility to infection in patients with the hyper-IgE syndrome.

Staphylococcal infections are in general more severe in patients at either end of the age spectrum and in patients with nutritional deficiency. Certain endocrine disorders, such as hypothyroidism, adrenal insufficiency and diabetes mellitus are associated with increased susceptibility to infection. Yotis & Fitzgerald (1974) found that the synthetic estrogen, diethylstilbesterol, administered in therapeutic concentrations had a significant anti-staphylococcal effect *in vivo* and *in vitro*. A similar effect was produced by other gonadal hormones, and it was suggested that staphylococci have hormone receptors on their cell surface.

Constantopoulos *et al.* (1972) have described two families with recurrent *S. aureus* infections. The serum of these patients had low levels of a specific leukophilic gammaglobulin fraction which has been reported to stimulate phagocytosis and has been named tuftsin. The role of this serum factor is uncertain.

Recently, Larson & Blades (1976) have shown that influenza virus incubated with human neutrophils *in vitro* inhibited the phagocytosis of staphylococcus by neutrophils. They suggested that the inhibitory effect of the virus on phagocytes might be related to the high incidence and severity of staphylococcal pneumonia in patients with influenza virus infection.

Summary

The success or failure of *S. aureus* as a pathogen depends upon the effectiveness of the armament of the host against the weapons of the bacteria. The bacterial weapons include extracellular enzymes and toxins, anti-phagocytic components of the cell wall, and for some strains at least a capsule which resists complement activation without antibacterial antibodies. The armament of the host includes the highly effective barrier of skin and mucous membranes, complement opsonins, antibacterial antibody opsonins, and phagocytic cells. Once opsonized staphylococci are usually efficiently engulfed and rapidly killed by polymorphonuclear neutrophils and macrophages. Investigations of normal cellular and humoral response to staphylococci have provided some insight into significant microbial and host factors in staphylococcal disease, and the study of these responses in patients with defective immunologic mechanisms has been especially valuable in increasing our knowledge of host response to these bacteria. More effective methods for prevention and therapy of staphylococcal disease will depend on simultaneous advances in knowledge of the molecular biology of the microbe and physiology of host defence mechanisms.

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