

## Role of the Spleen in Pneumococcal Infection

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### Summary

Complement and the spleen interact in host defense against the encapsulated *Pneumococcus*, responsible for most OPSI. Splenectomized patients lack splenic phagocytes specialized to clear bacteria coated with only small amounts of IgG from the bloodstream, and they are unable to mount a sufficient antibody response for liver macrophages to overcome the defect.

*King* and *Schumacher* (1) first clearly demonstrated the role of the spleen in normal host defense against bacterial infection when they reported a unique clinical syndrome characterized by fulminant onset of high grade bacteremia with encapsulated organisms, predominantly the pneumococcus. The common denominator in their cases, as in hundreds of other similar reports (2), was lack of the spleen either as a consequence of surgical removal or congenital absence. The importance of the spleen was thus only realized by its absence.

Since the pneumococcus is the organism most frequently associated with overwhelming post-splenectomy infection (OPSI) (2), it is reasonable to consider the components of normal host defense which are important in protection against this bacterium. Only then can we reasonably presume to understand the role of the spleen in preventing infection by this and other encapsulated organisms.

The pneumococcus has been the object of intense investigation since it was first discovered in 1888. As the most common etiologic agent of bacterial pneumonia, it was a leading cause of significant morbidity and mortality in the pre-antibiotic era. Thus, much investigative ef-

fort has been directed at how to both treat and prevent diseases caused by this microorganism.

The unique property of the pneumococcus and other encapsulated organisms is an amorphous polysaccharide coating which allows these bacteria to escape ingestion or phagocytosis by white cells. Pneumococci cannot be killed unless they are phagocytized, and their antiphagocytic capsule must first be neutralized. The proteins which prepare organisms for ingestion or phagocytosis are termed opsonins, and the principal opsonins of the pneumococcus are complement and antibody (4).

Complement was first described as a substance in fresh human serum responsible for lysis of red cells and certain bacteria. Subsequent investigation has uncovered at least 20 distinct proteins of the complement system, and lysis is probably not its most important biologic function. As these new complement proteins were merely numbered in order of discovery, C1 was designated the first component, C2 the second and so on.

Many of the proteins of the complement system are enzymes that circulate in an inactive state. Once activated, however, they "turn on" other complement components so that tremendous potential for amplification exists. Like a cascade, for every molecule of C1 activated, ten to a hundred molecules of C4 and C2 may be recruited. C4 and C2 then combine in turn to form another enzyme capable of activating thousands of molecules of C3.

The third component of complement (C3) is one of the most important proteins of the complement system. Activation of C3 results

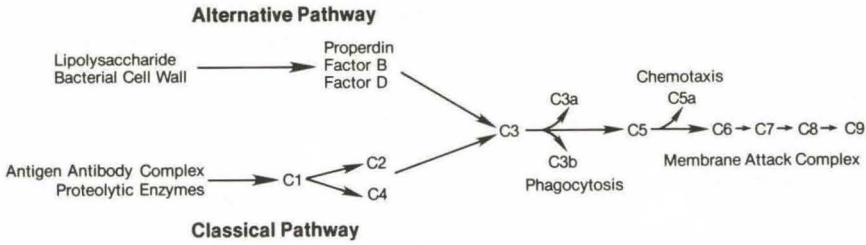


Fig. 1 Classical and alternative pathways of complement activation. See text for details

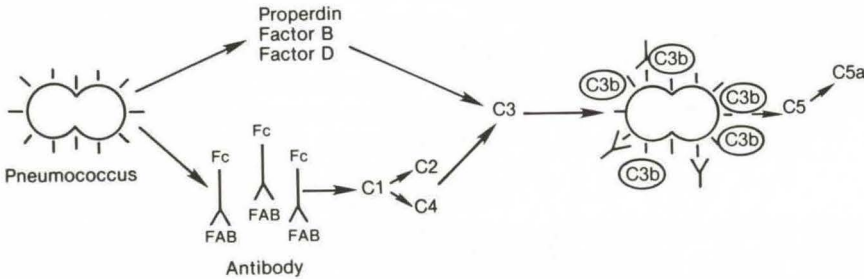


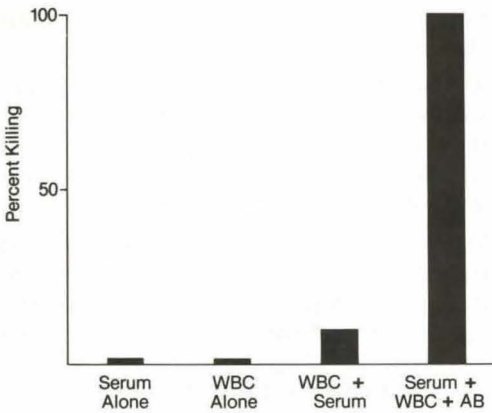
Fig. 2 Interactions of the pneumococcus with complement. See text for details

in cleavage of the protein into a small fragment C3a and a large molecular weight fragment C3b, which has the ability to bind many substances including bacteria, red cells and antigen-antibody complexes. White cells such as polymorphonuclear leukocytes and macrophages have surface receptors to bind C3b (5-8). Therefore, C3b acts as a bridge to bind bacteria (such as pneumococci) or red cells to phagocytes, white cells capable of ingesting bacteria. Only after white cells attach or bind to the bacteria does phagocytosis take place. C3b is particularly important in host defense against encapsulated organisms because it neutralizes the bacterium's antiphagocytic polysaccharide capsule. White cells then bind the organism, representing the first step in the process leading to ingestion and subsequent intracellular killing.

Activation of C3 occurs via either the classical or alternative pathway (Fig. 1). The clas-

sical pathway, including C1, C2, C4, was discovered around the turn of the century. It was not until the 1950s that an "alternative" means of activating C3 was first suggested by Pillemer. The components of the alternative pathway include factor B, factor D and properdin. The classical pathway is activated by antigen-antibody complexes as well as certain proteolytic enzymes, while the alternative pathway is activated by bacterial cell walls and lipopolysaccharides. The classical pathway is a much more efficient activator of C3 than the alternative pathway, and it has been postulated that the latter is phylogenetically older serving to transiently halt microbial invasion of the body until more specific (antibody and the classical pathway) defenses are mobilized.

The next component in the complement sequence is C5, activation of which also yields two fragments, the small C5a and the larger C5b. C5a dilates capillaries, causes white cells



**Fig. 3** Percent pneumococci killed after incubating  $5 \times 10^7$  type 2 organisms with either 10% normal human serum (complement),  $5 \times 10^7$  polymorphonuclear leukocytes (wbc), and/or type specific IgG antipneumococcal antibody (AB). No significant killing takes place unless white cells, complement, and antibody are all present.

to migrate toward C5a and activates white cells for phagocytosis. From this scheme, it is readily apparent why complement is an important mediator of inflammation. Complement proteins C6 through C9 form a membrane attack complex capable of putting holes in the cell membranes of red cells and certain bacteria, such as *N. gonorrhoeae* and *N. meningitidis*.

Thus, when a pneumococcus invades the body, complement is activated by the bacterial cell wall resulting in deposition of C3b on the organism (Fig. 2). In addition, C5a attracts white cells into the area and stimulates their readiness to ingest and destroy the pneumococcus. In fact, the presence of complement is critical to the normal host defense against pneumococci. Animals without complement consistently die after challenge with live pneumococci administered intravenously (9). Complement and white cells are not, however, sufficient in defense against the pneumococcus. The other crucial component is antibody.

The importance of antibody in host defense against pneumococcal infections was first re-

cognized by the observation that agglutinating antibodies appear after patients recover from pneumococcal pneumonia. In addition, immunized animals (ones that had formed antibody) were protected from challenge with live bacteria (3). We now know that antibody is a protein with two ends, an FAB and an Fc portion. The FAB end combines with whatever antigen the antibody is directed against, in the case of the pneumococcus, the polysaccharide capsular antigen. The other end of the antibody molecule, the Fc portion, is then free to bind to white cells. Only IgG, but not IgM, has Fc fragments which bind to white cells (5-8), thereby serving as a bridge between bacterium and phagocyte as well as signalling the phagocyte to ingest the bacterium. Once ingested, the pneumococcus is readily destroyed.

Normal host defense against the pneumococcus can be simulated in a test tube (Fig. 3). A specific number of pneumococci are added to a source of complement such as normal human serum with polymorphonuclear leukocytes and/or antibody. After incubation at  $37^\circ\text{C}$  for 60 minutes, the number of living remaining pneumococci are determined and percent killing calculated (Fig. 3). Only when white cells, complement and antibody are present together does efficient killing of pneumococci occur. After complement and antibody opsonize the pneumococci, white cells phagocytize and destroy the bacteria.

Complement and antibody actually work together to opsonize pneumococci. Recent studies conclusively demonstrate that human antibody directed against pneumococcal polysaccharide induces activation and binding of C3b to the surface of the organism, greatly enhancing the ability of white cells to kill pneumococci (10).

In summary, the important components of host defense against the pneumococcus are complement and antibody to opsonize, and phagocytes, such as polymorphonuclear leukocytes or macrophages, to ingest and kill.

How then are these components of normal host defense altered by absence of the spleen?

For all practical purposes, the complement

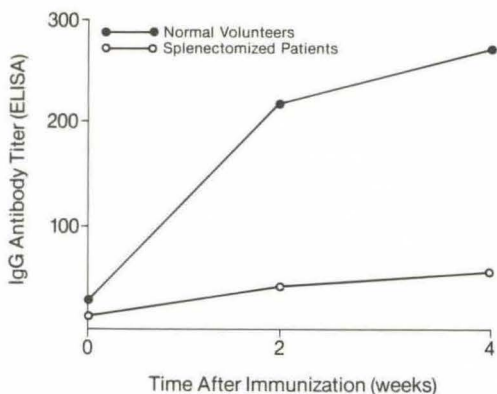


Fig. 4 Mean IgG antibody response to type 2 pneumococcal polysaccharide after polyvalent pneumococcal vaccination. Splenectomized patients produce antibody in lower titers and more slowly than do normal volunteers

system is intact in splenectomized patients. Although there are isolated reports of mild decreases in certain complement components such as properdin, serum from patients without a spleen opsonizes pneumococci as well as normal serum (11). The complement system has so much reserve that only after 99% depletion of serum C3 are severe opsonic defects noted (9). Thus, it is probably not a primary defect in the complement system which predisposes to OPSI.

Serum levels of type specific IgG and IgM anti-pneumococcal antibody are slightly decreased in splenectomized individuals. More importantly, however, after exposure to pneumococcal polysaccharide, serum levels of type specific IgG and IgM rise at a much slower rate and attain lower absolute levels than in normal controls (12) (Fig. 4). Since type specific antibody, especially IgG, is necessary for efficient phagocytosis and subsequent intracellular killing, inability to mobilize antibody may well contribute to development of OPSI. On the other hand, some other defect must also coexist since hypogammaglobulinemic patients have a similar disability in forming antibody and acquire infections with encapsulated organisms. Yet, in contrast to splenectom-

ized patients, they are not subject to catastrophic high grade bacteremia and shock.

Another proposed component of the normal host defense is tuftsin. This tetrapeptide has the ability to stimulate phagocytosis by white cells and is reportedly deficient in splenectomized patients (13).

The phagocytes themselves are also critical in normal host defense against the pneumococcus as they must ingest the opsonized pneumococcus in order to destroy it. Splenectomy does not impair the phagocytic function of circulating polymorphonuclear leukocytes and therefore, we must evaluate the fixed tissue macrophages of the reticuloendothelial system, which reside largely in the liver and spleen. Although the bulk of these are in the liver, splenic and hepatic macrophages may nonetheless behave differently.

Rate of clearance of opsonized particles from the bloodstream has been studied in normal subjects and splenectomized patients (14). In splenectomized patients, particles coated with C3b are cleared normally and then released back into the bloodstream (Fig. 5). As C3b only mediates binding to phagocytes not ingestion, clearance of C3b coated particles represents binding and release, probably as a result of inactivation of C3b.

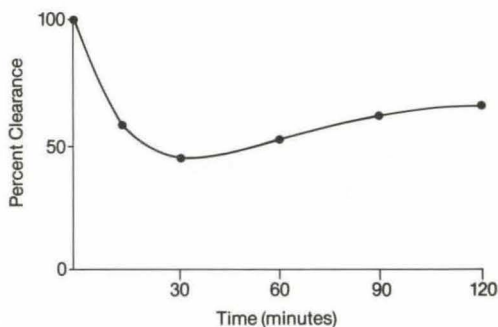
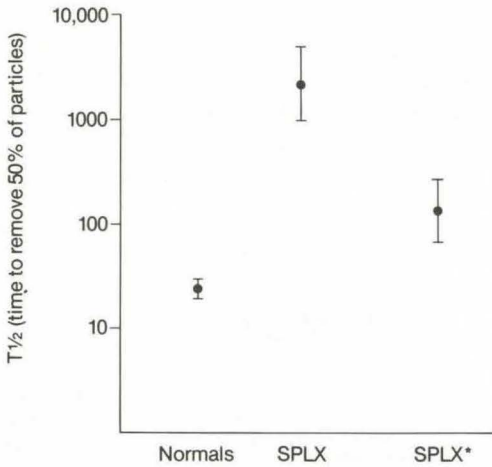


Fig. 5 Disappearance rate of intravenously administered C3b coated particles from the bloodstream of a splenectomized patient. Early rapid clearance followed by delayed resurgence suggests that in the absence of IgG antibody, initial tissue uptake of C3b-coated particles is reversible



**Fig. 6** Mean  $\pm$  SEM rate of clearance of IgG coated particles from the bloodstream of normal volunteers (NORMALS), splenectomized patients (SPLX) and splenectomized patients challenged with increased amounts of IgG antibody on the surface of indicator particles (SPLX\*). Splenectomized patients exhibit a marked defect in removing IgG coated particles from the bloodstream in the absence of increased amounts of antibody

In distinct contrast, IgG coated particles in splenectomized patients exhibit a 75-fold delay in clearance from the bloodstream after intravenous injection (mean 1/2-time to remove IgG coated particles is 47 minutes in normal controls compared with 3633 minutes,  $p < .001$ ) (Fig. 6). This clearance defect is corrected by increasing the amount of IgG coating the test particles and thereby shifting clearance to the liver. Thus, splenectomized patients are markedly deficient in their capacity to remove IgG coated particles from the bloodstream, especially when there are "low" numbers of IgG molecules coating the particles.

The unique splenic architecture probably explains the special ability of macrophages there to phagocytize IgG coated particles efficiently. As blood passes through the spleen and courses into the sinusoids, particles per-

colate slowly allowing ample time for intimate contact with splenic macrophages. On the other hand, the rapid flow of blood through the liver permits only brief contact of particles with hepatic macrophages. Increasing amounts of IgG on the surface of particles may nonetheless compensate for decreased contact time with hepatic macrophages.

What then are the implications of these observations in understanding the pathogenesis of OPSI? Recent studies have shown that within minutes after exposure to or colonization with encapsulated organisms, a transient bacteremia ensues (15). Presumably, complement is activated and C3b and any specific circulating antibody is bound to the bacteria. Unless a patient has been immunized against the organism, the low levels of antibody present require that splenic macrophages be the clearing agents of these bacteria from the bloodstream. In the absence of the spleen, however, the bacteria can circulate in the blood for prolonged periods of time. As pneumococci in log phase growth can divide every 90 minutes at a clearance rate of 3633 minutes, approximately 50 divisions are feasible, rapidly resulting in high grade bacteremia. In addition, the impaired ability to make type specific anti-poly-saccharide IgG after splenectomy renders hepatic macrophages unable to compensate for lack of the spleen, and overwhelming sepsis ensues.

Thus, postsplenectomy infection with encapsulated organisms such as the pneumococcus can be understood in terms of what is known about the normal host defense against these organisms. Splenectomized patients must face a double-edged sword when challenged by infection with encapsulated organisms. Not only are they missing the phagocytes or macrophages necessary to clear bacteria coated with small (or natural) amounts of IgG from the bloodstream, but they cannot mount a sufficient antibody response to allow the liver macrophages to compensate for the defect. Catastrophic onset of fulminant sepsis (OPSI) is the unfortunate result.

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