Host-Parasite Interaction in Staphylococcal Abscesses

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Review Article

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Staphylococci exhibit three distinct patterns of survival within abscesses. Some strains are destroyed rapidly whereas others persist for 2-3 months. Still others strains are eliminated, but only after a delay of 7-10 days (1,2). To solve the problem many investigators have studied staphylococcal abscesses and their host-parasite interaction.

In 1966 Kapral showed that when Staphylococcus aureus strains possessing clumping factor inoculated into the peritoneal cavity of mice, they are promptly clumped through the interaction of clumping factor and fibrinogen in the peritoneal fluid (3). During the ensuring inflammatory response, the clumped organisms became surrounded by thick layer of leucocytes, but phagocytosis was negligible because leucocytes in proximity to the cocci were quickly disrupted. Over the next 4 days the clumps became enclosed by connective tissue. The abscesses consist of a core of densely packed cocci, a broad zone of acellular debris, a zone of disintegrating leucocytes, a region of intact leucocytes and a vascularised connective-tissue capsule (1). The destruction of staphylococci did not result from phagocytosis or from the release of lysosomal substances, but rather from the production of bactericidal lipids (4,5).

The elimination of the cocci is mediated by two kinds of lipids (1-3). One consists of a pool of long-chain unsaturated, free fatty acids and the other is an unidentified lipid. The composition of the total lipid fraction remained fairly consistent with neutral lipids accounting for 90 per cent and phospholipids and glycolipid for 9 per cent and 1 per cent respectively (6). The lipids extracted from microbial or animal sources generally consist of 60-85 per cent phospholipid and glycolipid (7), thus the high neutral lipid content in abscesses is distinctly unusual. However, it should be noted that injured or hypoxic tissue culture cells have been found to contain high levels of triglycerides and free fatty acids (8). The unsaturated long chain fatty acids and the unidentified lipid collectively comprise about 17 per cent and <1 per cent of total lipids in abscesses respectively (9). The characteristic of the unidentified lipid is clearly distinguished from the fatty acids. Firstly, it is about 100 times more active against staphylococci than the most active unsaturated fatty acid (3). Secondly, it manifests differential activity which is expressed by a differential activity index greater than 10 (9). Thirdly, whereas, most active fatty acids are more active at an acid pH (10), the lipid exhibits no significant change in activity over the range pH 5.5-9.0. Fourthly, unsaturated fatty acids lose their bactericidal activity upon catalytic reduction because they are converted to their saturated counterparts. However, catalytic reduction of the lipid does not alter its activity. Finally, whereas unsaturated fatty acids tend to become more toxic for bacteria after oxidation or peroxidation, its activity is completely destroyed upon oxidation (9).

From histological section (6) of peritoneal abscesses of the experimental mice. Leucocytes containing cytoplasmic lipids droplets are first seen 4-12 hours after infection. These cells appear to be macrophages, and are widely scattered around the extreme periphery of the leucocyte layer. By 24 hours, sufficient lipid-laden cells have accumulated to form an almost continuous layer around the periphery to the developing abscesses. Two days later the leucocyte layer become enclosed by the connective tissue capsule, numerous small lipid droplets appear among the leucocytes located in the deeper portions of the region. After 4-7 days, droplets of lipid are present throughout the structure even in the core of the lesion which consisted of cocci and debris, but no intact leucocytes. However, the greatest accumulation of lipid is found among those leucocytes present just beneath the connective tissue capsule.

The site where the greater amounts of lipid are accumulated, suggest that the lipid production or accumulation is associated with cells which have recently entered the abscess. The investigators (6) found that the original resident macrophages failed to accumulate lipids, but a population of macrophages arriving subsequently accumulated substantial amounts of lipid in their cytoplasm. Peak levels of bactericidal activity coincided with maximal levels of neutral lipids which majority consist of a pool of free fatty acids and another unidentified lipid.

The activity by the lipids is activated by either live S. aureus or culture filtrates. After activations of abscess homogenates there is a 50 per cent decrease in amount of the triglycerides present and a corresponding increase in amount of free fatty acids (9). Suggestively that the hydrolysis of glycerides may be probably due to lipase activity (11), more than 99 per cent of S. aureus produce lipase (12). The activation may from the staphylococcal lipase itself. After activation the diglyceride and monoglyceride concentrations and the composition of
the fatty acid pool remain relatively unchanged, suggests that the glycerides may be the source of fatty acids initially present in the abscesses (9). However, the basis for activation is not clearly understood. *S. aureus* strains differ markedly in insensitivity to the lipid and strain sensitivity is correlated with survival within abscesses. Strains which are rapidly destroyed in abscesses are the most sensitive, whereas strains capable of long-term survival are the most resistant. Those strains which are destroyed after a delay are of intermediate sensitive to the lipid (2). By virtue of hydrophilic nature of bacterial capsules, capsule and non-capsulate *S. aureus* strains were comparatively studied (13) for their sensitivity to staphylococcal abscesses homogenates and the neutral lipid fractions derived from such material. The result was that the presence of a capsule seemed to reduce sensitivity, but the known resistant strain was not capsulate. This suggests that the mechanisms other than a capsule may determine sensitivity to the lipids.

A groups of investigators (14) found that freshly prepared culture filtrates also contained an enzyme capable of destroying the bactericidal activity of the lipids. It was called "fatty acid modifying enzyme" (FAME). The enzyme has a pH optimum between 5.5 and 6.0 and a temperature optimum of about 40°C. The enzyme activity is not affected by edetic acid or by the presence or absence of sodium and potassium ions. FAME can esterify without being an esterase operating in reverse. It can utilise methanol, ethanol, 1-propanol, 2-propanol, 1-butanol or cholesterol as substrates, but it more prefers cholesterol. *S. aureus* strains capable of producing the enzyme can synthesise it in trypticase soy broth and in chemically defined medium, but not necessarily in equal amounts. Ability of FAME to esterify fatty acids suggests that the enzyme may pay an important role in the host-parasite interaction, as the esterification of the bacterial fatty acids found in abscesses results in their inactivation (3). From an experiment (12) only *S. aureus* strains able to elaborate FAME where able to survive within host tissue. Those strains lacking FAME were rapidly destroyed when introduced into the tissue (14), but they did appear able to colonise mucosal surfaces.

From further study (15) found that when the abscess homogenates derived from *S. aureus* were treated with calcium ionophores the production of bactericidal lipid was stimulated, with properties indistinguishable from those previously unidentified lipids. The 2-monoglyceride is synthesised in abscess homogenates, but that this quickly isomerises to form the 1-monoglyceride. Both isomers are bactericidal. The 2-monoglyceride exhibited all the properties previously attributed to the unidentified lipid (15). Its bactericidal activity is neutralised by Ca++, licithin and *S. aureus* α toxin and is destroyed by oxidation or exposure to *S. aureus* FAME. Inositol 1, 4, 5-triphosphate can substitute for calcium ionophore in stimulating monoglyceride synthesis in abscess homogenates. This suggests that release of calcium from intracellular stores is required for monoglyceride production. The cell-free supernatant fraction of the abscess homogenate or the washed sediment does not result in the production of monoglyceride. However, production of monoglyceride is seen when the ionophore is added to the recombined fractions. This suggests that some soluble substance (s) in the supernate must act in concert with membranous components to synthesise the monoglyceride (15).

The fatty acid moiety of the monoglyceride pool consists primarily of palmitoleic acid and palmitic acid, whereas the pool of free fatty acids produced in abscess is composed of mainly oleic acid and palmitic acids, with smaller amounts of palmitoleic and linoleic acids. This is in keeping with the view that these two types of bactericidal lipid arise from different sources and through different mechanisms (15).

In conclusion, the host resistance to staphylococcal abscesses is majority due to bactericidal lipids. It does not depend on phagocytosis as previous described. The elimination of cocci is mediated by two kinds of lipids. One consists of a pool of long-chain unsaturated fatty acids and the other is 2-monoglyceride. The lipid production or accumulation is associated with leucocytes, particularly macrophages which have recently entered abscesses. The activity of unsaturated fatty acids is activated probably by staphylococcal lipase.

The bactericidal activity of both types of lipid is destroyed by an enzyme called "fatty acid modifying enzyme" (FAME) which is produced by resistant strains of *S. aureus*. Only those able to elaborate FAME are able to survive within host tissue. Strains lacking FAME are rapidly destroyed in abscesses. This interaction may explain in vivo bactericidal properties of tissue substances in the inflammatory process of staphylococcal abscesses. However, the mechanisms have to be studied further.
REFERENCES