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DEGRADATION OF ^{14}C-LABELED GROUP A STREPTOCOCCI AND MICROCOCCI IN MUSCULAR LESIONS IN THE MOUSE

In previous reports (1, 2) it has been shown that lysosomal enzymes derived from various populations of mammalian leukocytes failed to degrade ^{14}C-labeled group A streptococci *in vitro*. On the other hand, *Staphylococcus albus*, *Micrococcus lysodeikticus* and *Escherichia coli* were degraded to a large extent by leukocyte lysosomal enzymes. It was thus of interest to study the degradation of a variety of labeled microorganisms *in vivo* in inflammatory lesions in the thigh muscle of the mouse induced by the injection of heat-killed microorganisms. The results indicate that there may be a correlation between the degree of degradation of microorganisms by leukocyte lysates *in vitro* (1, 2) and the length of their persistence in lesion sites *in vivo*.

MATERIALS AND METHODS

Type 4 group A streptococci, strain K-43 A-variant streptococci, *S. albus*, *M. lysodeikticus* and *Streptococcus mitis* were labeled with ^{14}C (2) by growing the bacteria for 16 hr at 37 C in Brain-Heart Infusion broth (Difco), containing 1 to 2 mc/ml of D-glucose ^{14}C (2 to 4 mc/mmole). Normal Swiss mice received injections into the thigh of 0.1 ml of heat-killed labeled bacterial suspensions, containing approximately 2.5×10^6 count/min per 300 Klett units per ml. Labeled group A streptococci were also injected into mice which had previously been immunized with heat-killed streptococci (3). In some experiments, streptococci labeled with fluorescein isothiocyanate (4) were also injected i.m. and the fluorochrome label was detected in the tissues as described in ref. 4. The content of acid hydrolases in the mouse tissue and muscular lesions was determined as described in ref. 5. The mice were killed after different time intervals and the muscles at the lesion sites were homogenized in saline containing 0.1% Triton X-100. The radioactivity in the homogenates was determined in a Packard scintillation counter using toluene-Triton X-100 as scintillation fluid. The results were expressed as count/min per mg protein and calculated as the percentage of the total radioactivity remaining at the site of injection after different time intervals. Pieces of tissue obtained from the lesion sites were also fixed in glutaraldehyde and processed for electron microscopy by established procedures.

RESULTS AND COMMENT

In all experiments, except those with *M. lyso-*

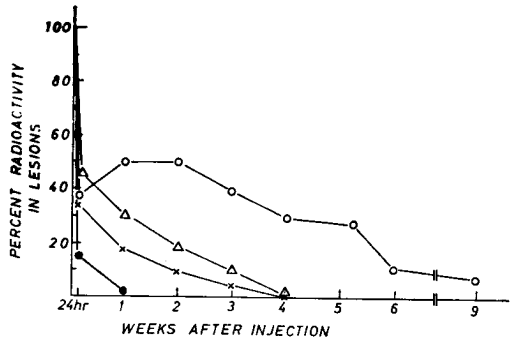

FIG. 1. Elimination of labeled group A streptococci, *Str. mitis*, *S. albus* and *M. lysodeikticus* from muscle lesion in the mouse. Results are expressed as the percentage of the radioactivity remaining in the lesion after the injection of ^{14}C-labeled bacteria. Each point on the graph represents an average of 10 mice. O, group A streptococci; △, *Str. mitis*; ×, *S. albus*; ●, *M. lysodeikticus*.

deikticus, a severe inflammatory reaction appeared at the site of injection within one to three days. The early lesions were characterized by muscle necrosis and by abscess formation rich in polymorphonuclear leukocytes, whereas the later lesions were rich in mononuclear cells. Fig. 1 shows that 24 hr after the injection of labeled microorganisms, there was a 50 to 85% decrease in the radioactivity of the injected muscle. The radioactivity due to *M. lysodeikticus* completely disappeared from the muscle within one week whereas approximately 50, 20 or 10% of the total radioactivity due to group A streptococci, *Streptococcus mitis* and *S. albus*, respectively, could be recovered from the injected muscle two weeks after the injection. By the fourth week no radioactivity due to *Str. mitis* and *S. albus* could be detected in the muscle while 5 to 10% of the radioactivity due to group A streptococci was still present in the injected site nine weeks after the injection. In other experiments it was found that no enhanced rate of elimination of group A streptococci occurred in mice which were previously immunized with streptococci. The sera of these mice had a high titer of agglutinins to streptococci, and their spleens, livers, peritoneal macrophages and lesion sites had two- to sevenfold elevated activity of lysozyme, acid phosphatase, N-acetylglucosaminidase and cathepsin D. Since fluorochrome-labeled streptococci and the A-variant streptococci (which lack a terminal N-acetylglucosamine from the C-polysaccharide) were found to be eliminated from muscular lesions at the same rate, it is assumed that the lack or removal of N-acetylglucosamine by leukocyte N-acetylglucosaminidase (6) does not contribute to a faster degradation of the streptococci. Similar results were obtained when ^{14}C-labeled streptococci and the A-variant mutant were injected i.m. Fig. 1 shows that 48 hr after the injection of group A streptococci, there was an unexpected increase in the total radioactivity at the lesion sites. This phenomenon was highly reproducible (the value given represents an average of 10 lesion sites). No such results were obtained, however, with the other bacteria employed.

Electron micrographs taken from lesions caused by group A streptococci showed the persistence for at least two weeks of numerous undegraded whole cells and intact streptococcal cell walls within phagocytic cells (Fig. 2). Under the same experimental conditions, no trace of *M. lysodeikticus* cells could be found. A full report on the *in vivo* degradation of *S. albus* and *S. aureus* in tissues of mice and rabbits and the nature of the bacterial cell constituents solubilized by leukocytes will be published elsewhere.

The data presented show that the microorganisms, previously found to be readily degraded *in vitro* (1, 2), were likewise quickly eliminated from muscular lesions (Fig. 1). These results are in accord with the findings on the long persistence of cell wall components of group A streptococci in tissues of rabbits (4, 7–9) and mice (10), the persistence of undegraded streptococcal cell wall components following phagocytosis (11) and the

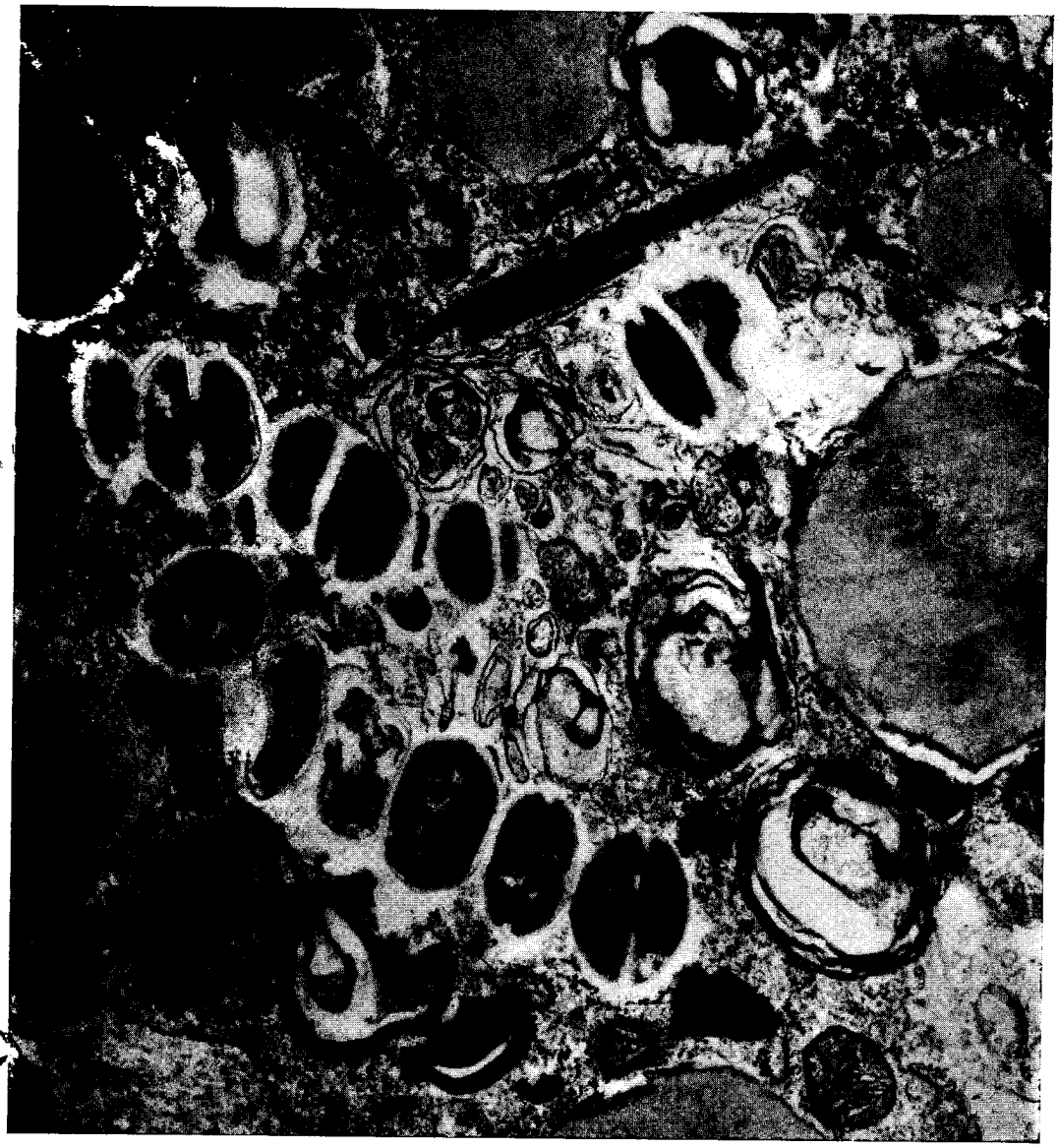

FIG. 2. A section of a macrophage in a mouse muscle lesion 14 days after the injection of heat-killed group A streptococci. Note the chains of streptococci within the cytoplasm of the phagocyte. Most of the cells appear intact. In a few streptococci plasmolysis is evident but the cell wall appears intact. × 39,600.

insignificant lysis of streptococci by leukocyte lysates *in vitro* (1, 2, 11). The finding of increased radioactivity due to group A streptococci in the inflammatory lesions 48 hr after injection (Fig. 1) is difficult to interpret at present. It may be postulated that undigested labeled bacteria, which were eliminated from the injected sites via the blood and lymph to the reticuloendothelial system (RES), were translocated within phagocytic cells back to the lesion sites in the muscle as previously described in detail (12–15). As to the mechanism

involved in the degradation of bacteria *in vivo*, the available data do not establish with certainty whether the elimination of the radioactivity from the injected sites (Fig. 1) represents enzymatic cleavage by leukocyte and tissue hydrolases *in situ*, or whether it is due to the elimination of the labeled bacteria by phagocytic cells, leaving the lesion sites to the RES. It is possible, however, that both mechanisms are functioning simultaneously.

Finally it is suggested that the lack of efficient degradation of bacterial wall components in inflammatory lesions may lead to granulomatous reactions giving rise to chronic sequelae (15).

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