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Oxygen-stable Hemolysins of Group A Streptococci VII. The Relation of the Leukotoxic Factor to Streptolysin S

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The capacity of certain streptococcal strains to kill leukocytes following phagocytosis was described by Levaditi [1] and by Wilson [2]. Wilson called this leukotoxic property of group A streptococci the leukotoxic effect. The leukotoxic property of the streptococcus was associated with very young cultures and could not be correlated with any known streptococcal product. More recently, Bernheimer et al. [3] showed that the majority of leukotoxic group A strains produced NAD glycohydrolase (NADase), while most of the nonleukotoxic strains failed to produce this enzyme. He therefore postulated that NADase was probably identical with the leukotoxic factor. Purified preparations of NADase, however, failed to exert any toxic effect on leukocytes. It was further postulated that streptococci produced the enzyme intracellularly following phagocytosis, and thus impaired the metabolic activity of the leukocyte, which resulted in its death. Ginsburg and Grossowicz [4] demonstrated that washed group A streptococci harvested from young cultures possessed a cell-bound hemolysin (CBH) capable of hemolyzing red blood cells. Later, it was shown that CBH produced cytopathic effects in leukocytes, Ehrlich ascites tumor cells, and a variety of other mammalian cells [5–7]. Under ordinary conditions, no extracellular hemolysin could be detected in supernatant fluid of streptococcal suspension possessing CBH activity. The hemolytic factor was, however, released into the surrounding medium by RNA, serum albumin, tween, and triton [8]. The hemolytic activity thus released was recognized as streptolysin S (SLS) [8, 9]. More recently, it has been shown that the CBH is identical with SLS [10]. The purpose of the present communication is to show that the leukotoxic effect of group A streptococci on leukocytes is due to the cell-bound hemolysin (streptolysin S).

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Materials and Methods

Streptococcal strains. The streptococcal strains employed are listed in table 1. They were typed by the precipitin method of Swift et al. [11] (M-strains), with the exception of strain C203S and C203U, which were typed by agglutination only (T-strains). Strain 41448 (type 12) was virulent for mice; the others were avirulent for mice. The streptococcal strains were cultivated either in Todd Hewit broth supplemented with 1% horse serum (for NADase activity) or in brain heart infusion medium (BHI) (Difco).

Cell-bound hemolysin. The cell-bound hemolysin was assayed according to Ginsburg et al. [10]. The bacterial cells harvested at different phases of growth were washed with saline buffered with 0.025 M phosphate, *p*H 7.4, (PBS), resuspended in buffer, and adjusted to 100 Klett units with a 540 filter. Such streptococcal suspension contains approximately 10^9 cells/ml. The streptococcal suspension (0.5 ml) was incubated for 5 min at 37 C with 0.1 ml of an activation mixture (AM) (10 mg/ml each of glucose, $MgSO_4 \cdot 7\ H_2O$, and 1.3 mg/ml of cysteine-free base). Following incubation, the streptococcal suspension was serially diluted in buffer (final volume of 0.5 ml), and 0.5 ml of a 2% suspension of group O human red blood cells in the same buffer