SEROLOGICAL CROSS-REACTIVITY BETWEEN A RIBITOL TEICHOIC ACID AND A COMPONENT OF ESCHERICHIA COLI[1]

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Received May 28, 1963

Teichoic acids are polymers of ribitol phosphate or glycerophosphate containing esterified D-alanine and glycosidically linked sugars (Armstrong et al., 1958). Varying amounts of ribitol teichoic acids have been found in the walls of many Gram-positive bacteria, while the glycerol containing polymers have been reported to occur both in the wall and in the interior of these micro-organisms (Armstrong et al.,1959). More recently, it has been claimed that in at least two instances, the "intracellular" glycerol teichoic acid is in fact located between the membrane and the wall of the cell (Hay et al., 1963). The teichoic acids have been shown to be antigenic and serological cross-reactions have been demonstrated between polymers isolated from various sources (Baddiley and Davison, 1961; Haukenes et al., 1961; Sanderson et al., 1961; Morse, 1962). In staphylococcal ribitol teichoic acids, the immunological determinant groups have been reported to be alpha- and/or beta-

[1]Supported by USPHS grant No. AI 04789-01 from the National Institute of Allergy and Infectious Diseases, and a grant from the Sigrid Juselius Foundation, Helsinki, Finland.

N-acetylglucosamine (Haukenes et al., 1961; Sanderson et al., 1961). The chemical and immunological studies on teichoic acids have in general been confined to Gram-positive organisms.

In this communication, we wish to report an immunological cross-reaction between a purified ribitol teichoic acid and an antiserum prepared against disrupted cells of Escherichia coli B.

During the course of another investigation (Lahti et al., 1963), rabbits were immunized with disrupted cells of E. coli B. The details of the preparation of the sera (which in immunoelectrophoresis revealed 24 antigens in E. coli B) will be reported elsewhere (Vainio and Lahti, in preparation). When the four individual rabbit antisera which were available for this investigation were tested with the double diffusion precipitation technique (DDP) against ribitol teichoic acid, which had been purified from the walls of Staphylococcus aureus H according to Baddiley et al. (1962), a distinct precipitation band was obtained with all four sera[1].

Several antigen preparations were tested against an anti-E. coli B serum employing the micro-double diffusion precipitation technique of Wadsworth (1957). The results are summarized in Fig. 1. The serum which gave 7 to 8 precipitation

[1]The absence of contaminating bacteria in the cultures used both for the preparation of the E. coli B antigens and the teichoic acid was ascertained by careful bacteriological examination. Blood drawn from the rabbits shortly after the first antigen injection was negative when tested against the ribitol teichoic acid. No signs of bacterial infections were observed in the animals prior to or during the immunization. Furthermore, the possibility that such an infection could have given rise to the antibodies against the polymer could be disregarded since the sera of 16 other rabbits which had simultaneously been immunized in the same room for other purposes, did not react with the teichoic acid in DDP.

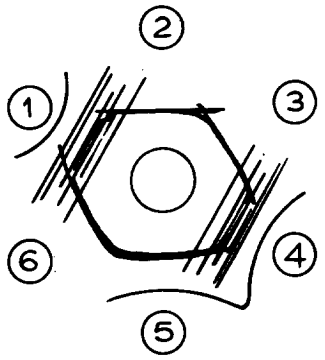

Fig.1. Schematic illustration of DDP bands formed between a rabbit anti - E. coli B serum and various antigens: 1) and 4) disrupted cells of E. coli B, 2) extract from E. coli B cells (see text), 3) and 6) teichoic acid from the walls of S. aureus H, 5) disrupted cells of S. aureus H.

bands with disrupted E. coli B cells, revealed two antigens in a similarly prepared mixture of S. aureus H antigens. When staphylococcal teichoic acid reacted with the serum, only one band was observed. It gave a reaction of identity with one of the two bands obtained with the complete set of S. aureus H antigens. The same band appears to give a reaction of partial identity with one of the bands seen with disrupted E. coli B cells. The uncertainty at this point is caused by the abundance of the precipitation bands formed between the serum and the components of E. coli B. The second band present in disrupted staphylococci but absent from the teichoic acid preparation, gave a strong reaction of identity with one of the E. coli B precipitation bands. A fraction prepared from E. coli B by extraction of the disrupted bacteria with hot dilute acid, followed by precipitation with acetone (Lancefield, 1933) produced a band in DDP, which appeared to give a reaction of partial identity with the teichoic acid band.

In another experiment, the homogeneity of the teichoic acid band was examined by means of immunoelectrophoresis. Only one component was revealed with this technique. In an attempt to identify the immunological determinant involved in this pre-

cipitation reaction, experiments using a modification (Lahti et al., in preparation) of the conventional inhibition technique (Kabat and Mayer, 1961) were conducted. It was shown that an excess of N-acetylglucosamine inhibited the formation of the specific precipitation while equally high concentrations of several other sugars did not inhibit the reaction (Lahti et al. in preparation). Our purified product was, as is generally the case (Baddiley et al., 1962), contaminated by a small amount of nucleic acid. Other fractions obtained from the staphylococci, containing the bulk of the bacterial nucleic acids failed to give the precipitation band. Treatment of the teichoic acid with ribonuclease, deoxyribonuclease or trypsin had no effect on the precipitation reaction.

It seems reasonable to conclude that the precipitation band formed by the teichoic acid and E. coli antiserum actually results from an interaction between the polymer and an antibody formed against the antigenic determinants present in the teichoic acid. The possibility that an impurity in the teichoic acid preparation would be responsible for the immune precipitation is highly unlikely. The polymer which had been obtained in a reasonably well purified form, is known to be a good antigen which reacts even at a low dilution (Haukenes et al., 1961). Ribonucleic acid, the only impurity present in measurable amounts is a notoriously poor immunogen, and its participation in the precipitation can be ruled out by the experiments cited above.

The nature of the immunogen present in E. coli B is obscure. For the reasons given earlier in this paper, we are confident that a component (or components) in E. coli is (are)

responsible for the appearance of antibodies reacting with the staphylococcal teichoic acid, and that a bacterial contamination can be ruled out. The decision whether E. coli cells contain compounds structurally similar to the teichoic acids, or whether the immunizing agent is an unrelated substance which contains the same determinant groups, must await further experimentation.

The identity of the second staphylococcal antigen which cross-reacts with E. coli, as well as the chemical nature and localization of the immunogens in E. coli B are under investigation.

The authors are indebted to Mrs. K. Nousiainen and Miss E-M. Kuure for valuable technical assistance.

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