

Specificity of human serum antibodies against endotoxin from *N. gonorrhoeae*

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The polysaccharide component of endotoxin from Gram-negative bacteria such as the *Salmonellae* is the carrier of several antigenic factors. These structures determine the specificity of antibodies against the endotoxin and the O-antigenic classification of the corresponding bacterium (Lüderitz, Staub, and Westphal, 1966). Our knowledge of endotoxin from gonococci is limited in comparison with endotoxins from members of the *Enterobacteriaceae* which are well characterized. However, it has been shown that sera from rabbits immunized with gonococci contain antibodies against different antigenic factors carried by the polysaccharide component of endotoxin from the bacterium (Mæland, 1969b; Mæland, Kristoffersen, and Hofstad, 1971). Results have been reported indicating that antibodies of various specificities may also be involved in the reaction of human serum with endotoxin from gonococci (Mæland and Larsen, 1975).

In this study experiments have been performed to elucidate further the specificity of human serum antibodies against the polysaccharide component (determinant *a*) of endotoxin from gonococci. Sera from patients with gonorrhoea were tested for antibody activity against determinant *a* from three different strains of gonococci before and after treatment with mercaptoethanol. The sera were examined unabsorbed and after absorption with endotoxin from the same strains.

Material and methods

SERA

Sera were obtained from patients treated for gonorrhoea at the Outpatients Clinic for Venereal Diseases, the University Hospital, Bergen. The diagnosis was verified bacteriologically. Fresh sera were stored frozen. The sera were heat-inactivated at 56°C. for 30 min.

Sera were examined before treatment with mercaptoethanol (untreated serum) and after treatment (ME-treated serum). Equal volumes of serum diluted 1 in 2

and 0.2 M ME in phosphate-buffered saline pH 7.2 (PBS) were mixed. The mixture was incubated at 37°C. for 30 min. and tested without alkylation.

PREPARATION OF ANTIGEN

N. gonorrhoeae strains 8551, V, and VII were cultured and harvested as reported (Mæland, Wesenberg, and Tønder, 1973). The phenol-water endotoxin was prepared by extraction of bacterial whole cells with phenol-water and purified by ultracentrifugation and washing as earlier (Mæland, 1968). The final preparations were lyophilized.

Lyophilized material was treated with NaOH and digested with pronase in order to obtain the *a* determinant from each strain (*a*-8551, *a*-V, and *a*-VII), according to a procedure described previously (Mæland, 1969a). The preparations were stored frozen and were used for the sensitization of sheep erythrocytes.

SENSITIZATION OF ERYTHROCYTES

Sheep erythrocytes were sensitized with the preparations containing the various *a* determinants as previously described (Mæland and Larsen, 1971). Sera from patients with gonorrhoea were examined by chequerboard titration against sensitized erythrocytes to determine the sensitizing activity of the preparations. From eight to sixteen times the least amount giving the maximum titre of the sera was used for the sensitization.

INDIRECT HAEMAGGLUTINATION (IHA) TEST

Sera were absorbed with unsensitized sheep erythrocytes before testing. Two-fold dilutions of the sera were prepared in 0.05 ml. volumes in PBS. An equal volume of 0.5 per cent. suspension of the sensitized sheep erythrocytes in PBS was added. Incubation and reading of the agglutination were performed as previously described (Mæland and Larsen, 1971).

ABSORPTION OF SERA WITH ENDOTOXIN

1 ml. of diluted serum (1 in 4) was absorbed with 0.5 mg. of the endotoxin preparation. This quantity was *in excess* of that required to deplete serum of antibodies against the corresponding preparation. The mixture was incubated at 20°C. for 1 hr, 4°C. for 20 hrs, and spun at 2,000 × G for 15 min. The supernatant was examined by the IHA test.

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Results

Sera from patients with gonorrhoea were examined by the IHA test for antibody against determinant *a* from strains 8551, V, and VII. Sera showing comparatively high titres of antibodies against all three *a* determinants both before and after treatment with ME were selected for further experiments. Samples of these sera were absorbed with endotoxin from the same strains and again examined by the IHA test. The results obtained with five sera are shown in the Table as representative of the experiments performed. It is seen that treatment of the sera with ME either reduced or had no effect on the titres obtained.

TABLE *Sera from patients with gonorrhoea examined by indirect haemagglutination for antibodies against determinant a of endotoxin from N. gonorrhoeae strains 8551, V, and VII. Titres of untreated and ME-treated sera (in parenthesis) before and after absorption with endotoxin are shown*

Serum no.	Endotoxin used for absorption	Erythrocytes sensitized with		
		a-8551	a-V	a-VII
1	None	128(128)	128(64)	256(64)
	8551	<4(<4)	32(32)	64(64)
	V	<4(<4)	<4(<4)	16(16)
	VII	32(16)	16(8)	<4(<4)
2	None	64(16)	32(32)	32(16)
	8551	<4(<4)	<4(<4)	8(<4)
	V	32(<4)	<4(<4)	8(<4)
	VII	64(<4)	<4(<4)	<4(<4)
3	None	32(16)	16(16)	16(16)
	8551	<4(<4)	8(<4)	8(<4)
	V	16(<4)	<4(<4)	<4(<4)
	VII	16(<4)	<4(<4)	<4(<4)
4	None	64(32)	32(32)	32(32)
	8551	<4(<4)	8(8)	16(8)
	V	16(16)	<4(<4)	8(<4)
	VII	32(32)	8(<4)	<4(<4)
5	None	128(32)	128(64)	256(32)
	8551	<4(<4)	64(16)	64(32)
	V	32(<4)	<4(<4)	128(16)
	VII	32(16)	<4(<4)	<4(<4)

Absorption with endotoxin of sera not treated with ME

After absorption of the untreated sera with endotoxin from any of the strains used the activity of antibody against the corresponding *a* determinant was always reduced to titres less than 4 (Table). However, all the absorbed samples demonstrated antibody activity against one or both of the other *a* determinants. Thus a pattern of reactivity emerged for the various sera under investigation. All five sera listed in the table had their own special pattern of reactivity when examined untreated. In absorption experiments using higher concentrations of the endotoxins the same results were obtained.

Absorption with endotoxin of sera treated with ME

Testing of the same sera after treatment with ME

gave results which resemble those obtained before treatment (Table). Also the activity in the IHA test of the treated sera resulted in a characteristic pattern of reactivity when unabsorbed and absorbed samples were tested. However, none of the absorbed samples of sera Nos 2 and 3 showed antibody activity against any of the *a* determinants. It appears from the table that the pattern of reactivity of serum No. 1 was the same before and after treatment with ME. However, the pattern of reactivity of the other sera was changed after the treatment. Sera Nos. 2 and 3 showed identical patterns of reactivity. These sera had different patterns before treatment with ME.

The results obtained indicate that the activity against determinant *a* is due to antibodies of various specificities in the sera tested.

Discussion

Sera from patients with gonorrhoea were used in order to study the specificity of antibodies against the polysaccharide component (determinant *a*) of endotoxin from *N. gonorrhoeae* strains 8551, V, and VII. The sera were examined by the IHA test before and after absorption with the endotoxins. The sera were also examined after treatment with ME in order to see whether this would change the pattern of reactivity exhibited by the untreated sera. The antibodies measured by the IHA test were present in comparatively low titres, as found earlier with sera from other patients with gonorrhoea (Mæland and Larsen, 1971, 1975). Nevertheless, with this reservation in mind, it is felt that the results obtained serve to elucidate the specificity of the antibodies involved.

The sera under investigation showed activity in the IHA test against all three *a* determinants both before and after treatment with ME. Absorption of the sera with any of the endotoxin preparations affected the antibody activity. Antibodies against the *a* determinant used for the absorption were always removed. However, all the absorbed samples of untreated sera and the majority of those of ME-treated sera, showed antibody activity against one or both of the other *a* determinants. Different results were obtained with two of the sera when tested after treatment with ME. Absorption of these sera with any of the endotoxin preparations removed the antibodies against all three *a* determinants. The results indicate that the sera contained antibodies of various specificities against the *a* determinants used. All five sera showed different patterns of reactivity when examined before treatment with ME. This observation puts further emphasis on the multispecificity of antibodies that may be present in sera from patients with gonorrhoea. Variation and strain specificity of human antibodies have also been suggested by Glynn and Ward (1970) in bactericidal tests with gonococci, and by Watt, Ward, and Glynn (1972) in an agglutination

test using latex particles sensitized with lipopolysaccharide from gonococci. It has been noticed that sera from patients with gonorrhoea may show antibody activity against the lipopolysaccharide from different strains of gonococci, but not to the same degree (Mæland and Larsen, 1975; Ward and Glynn, 1972). Fletcher, Miller, and Nicol (1973) demonstrated that a higher percentage of human sera show antibody activity in tests with lipopolysaccharide from several strains of gonococci than with antigen from a single strain. These observations may be explained by the present demonstration of multispecificity of human serum antibodies against the *a* determinants.

Treatment with ME resulted in an altered pattern of reactivity of four of the five sera tested. It appears therefore that the pattern of reactivity of the ME-resistant antibodies may differ from that of the ME-sensitive antibodies in the same serum. Earlier investigations have shown that the majority of sera from healthy subjects contain 'normal' antibodies of the IgM class against the *a* determinants used in this study (Mæland and Larsen, 1971). It is not known which of the sera tested contain normal antibodies against these antigens in addition to antibodies elicited during the infection with gonococci. However, it is conceivable that normal antibodies, when present, may influence the pattern of reactivity of sera from patients with gonorrhoea when examined without pre-treatment with ME. In order to test this hypothesis experiments should be done using serum and antigen from the strain of gonococcus isolated from the patient.

The results of this study indicate that a complex mosaic of antibody-combining sites is carried by the polysaccharide component of endotoxin from gonococci. Clearly, these structures may vary from one strain to another. This is in agreement with earlier findings in experiments with rabbit antisera against strains 8551, V, and VII (Mæland, 1969b; Mæland, Kristoffersen, and Hofstad, 1971). In these experiments six different antigenic factors were detected, and were shown to occur in various combinations among different strains of gonococci. Whether the *a*

factors and the structures with which human antibodies combine are identical remains a challenge for future studies.

Summary

Sera from patients with gonorrhoea were used to study the specificity of human antibodies against determinant *a* of endotoxin from gonococcal strains 8551, V, and VII. The sera were tested by an indirect haemagglutination technique before and after absorption with endotoxin from the same strains. The sera were used untreated and treated with mercaptoethanol (ME). The untreated and ME-treated sera showed antibody activity against all the *a* determinants when examined unabsorbed. After absorption with any of the endotoxin preparations the sera usually demonstrated antibody activity against one or both of the other preparations. The pattern of reactivity thus observed differed from one serum to another. Treatment of sera with ME resulted in an altered pattern of reactivity of four of the five sera. The results indicate multispecificity of human antibodies against the *a* determinants.

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