CONTROL BY FACTORS DISTINCT FROM THE S TRANSFORMING PRINCIPLE OF THE AMOUNT OF CAPSULAR POLYSACCHARIDE PRODUCED BY TYPE III PNEUMOCOCCI

By COLIN M. MacLEOD, M.D., and MARJORIE R. KRAUSS

(From the Department of Microbiology, New York University College of Medicine, New York)

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Mutants of several pneumococcal types have been described which form smaller amounts of capsular polysaccharides than the parent or normally encapsulated strain from which they derived. S transforming principles from these mutants confer on certain R strains of pneumococcus the capacity to produce amounts of polysaccharide characteristic of the S mutants. It has been suggested that the transforming principles of strains showing differences in polysaccharide synthesis bear a relation to each other similar to that of the genes of an allelic series (1–3).

In discussing the mechanisms involved in differences in polysaccharide formation it was suggested earlier (1) that variation might depend not only on "allelic" differences in the S transforming principles but also on some other system in the cell capable of modifying polysaccharide production. The present paper is concerned with the demonstration that polysaccharide formation can be modified by factors distinct from the S transforming principle. It has been shown that encapsulated pneumococci may possess and reduplicate normal S transforming principle and yet produce much less polysaccharide than would be expected if the nature of the S transforming principle were the sole determinant.

Materials and Methods

Transformation Reactions.—The preparation of the transforming extracts and the technique for carrying out the reactions were the same as described previously (1).

Strains of Pneumococci.—Type I strain 519/43/41° and R variants derived from it: Type I strain 519/43/41° was obtained originally by Dr. Robert Austrian from Dr. Erna Mørch-Lund of the State Serum Institute in Copenhagen. It had been transferred for 300 subcultures in serum broth at 41°C. in Dr. Mørch-Lund's laboratory and was reported (4) to have lost its power to ferment salicin within an incubation period of 24 hours, though its other fermentative capacities were unchanged. Five R variants were selected from strain I/519/43/41° by Dr. Austrian after 6 serial subcultures in broth containing type I antiserum. Cultures of the R variants were streaked on blood agar plates in this laboratory and a number of single colonies picked and transferred to broth for use in transformation reactions. Two of these R strains are referred to in the present paper as I-R6 and I-R7.

II-R36NC: A colonial variant of the R strain, R36, used in previous studies (1, 3). R36 was derived originally from type II strain D39S.

III-A66S: A fully encapsulated, "normal" strain of pneumococcus type III which has been used extensively in transformation studies.

Measurement of Capsular Polysaccharide.—Type III pneumococci were incubated in neopeptone-meat infusion broth at 37°C. for 8 hours and then placed in an ice bath to stop growth. Samples were removed for measurement of the viable count, bacterial nitrogen per milliliter, and the amount of capsular polysaccharide (SIII) per milliliter. An incubation time of 8 hours was chosen to avoid autolysis which would interfere with all of the above measurements except that of SIII.

The viable count was determined by using poured blood agar plates containing appropriate dilutions of the cultures.

Bacterial nitrogen was measured by micro Kjeldahl technique. The broth culture was heated at 65°C. for 30 minutes in a water bath to prevent autolysis, the pneumococci sedimented by centrifrigation, and then washed three times in saline before digestion.

For measurement of SIII production an aliquot of unheated broth culture was lysed by addition of sodium desoxycholate in a final concentration of 0.05 per cent. Complete clearing of the bacterial suspension occurred within 5 to 10 minutes at room temperature. SIII was estimated photometrically by measuring the absorption caused by antigen-antibody precipitates in the presence of excess antibody according to the technique of Bernheimer (5). To 0.5 ml. of an appropriate dilution of culture lysate in phosphate-buffered saline, pH 7.0, there was added 0.5 ml. of a 1:5 dilution of type III antipneumococcal horse serum[1] that had been absorbed with an extract of sonically disrupted R cells derived from type III pneumococcus, in order to remove antibody reacting with the somatic constituents of pneumococci.[2] After 60 minutes in a water bath at 37°C. the precipitate was resuspended by shaking and the optical density was read against a broth blank in a Beckman DU spectrophotometer at 650 mμ., employing microcells with a light path of 1 cm. The optical density was converted to micrograms SIII per milliliter by comparison with a standard curve prepared by use of a secondary standard of SIII having a purity of 60 per cent as compared with a highly purified preparation of SIII (6).

EXPERIMENTAL

Transformation to Type III of Different R Strains Derived from Type I Strain 519/43/41°.—Transformation reactions were carried out using transforming principle (TP) from a fully encapsulated "normal" strain of type III pneumococcus, IIIS-A/66. The IR strains employed were selected from strain I-519/43/41° by growth in broth containing type I antiserum. Five R strains were streaked on the surface of blood agar plates. Twelve R colonies, among them I-R6 and I-R7, were picked from the five strains, replated on blood agar and single colonies transferred to broth for use in transformation reactions. In the presence of the normal IIIS transforming principle, transformation to type III occurred readily with all of the IR strains that were tested. The transformed organisms from a single transformation tube gave rise to colonies that varied in size but were smaller than colonies obtained by transformation

[1] Type III antipneumococcal horse serum was provided by the Bureau of Laboratories, New York City Department of Health.

[2] The absorbed antiserum was prepared by Dr. A. W. Bernheimer.

of other R strains, such as R36NC, with extracts of the normal type IIIS strain. It should be noted that heretofore the IIIS colonies obtained by transformation reactions have resembled in size the colonies of the S organisms from which the transforming principle was prepared.

Presence of Normal Type III Transforming Principle in Transformed R Organisms That Formed Small IIIS Colonies.—Because of the small size of the IIIS colonies produced by the IR strains that had been transformed to type III by a normal IIIS transforming extract, it was of interest to prepare extracts from these transformed strains to determine whether they contained the normal type III transforming principle or whether an alteration had taken place in the principle upon residence and reduplication within the IR strains.

TABLE I

Presence of "Normal" Transforming Principle in Cells Producing Small Amount of SIII

Source of type III transforming principle	R strain transformed	Size of colonies formed by transformed organisms
IIIS-A66 (normal IIIS strain) naturally occurring	IIR36NC (normal R strain)	Large
" "	I-R6 (atypical R strain)	Small
" "	I-R7 (atypical R strain)	Small
IIIS-IR6 (small colony) obtained by transformation	IIR36NC (normal R strain)	Large
" "	I-R6 (atypical R strain)	Small
" "	I-R7 (atypical R strain)	Small

Normal IIIS transforming principle was applied to R strains I-R6 and I-R7. Twenty-four hours later a loopful of culture from each transformation tube was streaked on blood agar and after incubation 2 colonies of transformed organisms from each of the IR strains were selected. These 4 transformed strains of IR were then grown in broth and transforming extracts prepared from each of them and applied to strains I-R6 and I-R7 and to a strain of IIR, R36NC. Table I shows the results obtained with TP prepared from one of the transformed IR strains, and with TP from a normal strain of type IIIS. The results with the transforming principles from the other 3 transformed IR strains were identical with those shown in Table I for strain IIIS-IR6.

From the observations presented in Table I it is apparent that although the transformed IR strains produce small IIIS colonies, and have the appearance of organisms containing a mutated transforming principle, nonetheless, they actually contain the normal IIIS transforming principle, which, in the transformed IR strains, is not expressed phenotypically, but when applied to a "normal" R strain (II-R36NC) results in a transformed IIIS strain very similar

to that resulting from application of a TP derived from the normal strain of type III (A66).

The property of producing less than the normal amount of SIII appears to be stable, since cultures of transformed strain IIIS-IR6 in fluid or on solid medium maintain this characteristic upon repeated subculture and after mouse passage.

Quantitative Measurement of Production of Type III Polysaccharide by Normal and Transformed Strains.—In order to prove that the size of the colonies of the transformed IR organisms is due to a reduction in capsular polysaccharide

TABLE II

Polysaccharide Production of Normal and Transformed Strains Measured after Growth in Broth at 37°C. for 8 Hours

Strains of pneumococcus	Description	Viable count/ml.	SIII/ml.	Bacterial N/ml.	μg. SIII/μg. bacterial N	Mouse virulence*
			μg.	μg.		*ml. of culture*
IIIS-A66	Naturally occurring normal IIIS strain	2.4×10^8	36.3	10.0	3.63	10^{-8}
IIIS-R36NC	Transformed strain. Normal IIR strain transformed by TP from IIIS-A66	5.5×10^8	29.4	11.4	2.58	10^{-8}
IIIS-IR6	Transformed strain. IR strain transformed by TP from IIIS-A66	2.2×10^8	7.86	6.6	1.19	10^{-1}
IIIS-R36NC	Transformed strain. Normal IIR strain transformed by TP from IIIS-IR6	1.5×10^8	24.4	8.3	2.94	10^{-8}

* Volume of 18 hour broth culture causing death of mice.

production per bacterial cell and not merely a reflection of slower bacterial multiplication, the production of SIII was measured in young broth cultures after lysis by sodium desoxycholate. These measurements are recorded in Table II.

From Table II it can be seen that the transformed atypical IR strain (IIIS-IR6) produced only one-third as much SIII per microgram bacterial N as the fully encapsulated, normal strain IIIS-A66, and less than one-half as much SIII as the transformed normal IIR strain. However, despite the small amount of SIII produced by the transformed strain IIIS-IR6, the transforming principle prepared from it confers on the normal IIR strain the capacity to produce as much SIII as does the TP from the normal type III strain.

A modest decrease in the amount of SIII formed by transformed strains of

IIR36NC as compared with the naturally occurring strain IIIS-A66 has been observed consistently in the present and in previous experiments. The difference may depend upon technical factors, but more likely it is due to a lower ability of the transformed strain IIIS-IIR36NC to form polysaccharide even though it contains the normal IIIS transforming principle. If the latter is the correct explanation, then the markedly diminished capacity of strain IIIS-IR6 to form SIII is not so unique as might appear at first glance but represents rather an exaggeration of a defect which is also present in strain IIR36NC.

In Table II is also shown the virulence for mice of the four strains. The three strains that produce large amounts of polysaccharide are highly virulent, whereas strain IIIS-IR6 which produces a markedly reduced amount of SIII is avirulent.

DISCUSSION

The experiments reported in the present paper demonstrate that alterations in capsular polysaccharide formation by pneumococci may depend not only on the nature of the S transforming principle present in the cells but also on cell factors distinct from the S transforming principle.

In the case described the cells contain the "normal" transforming principle, yet synthesize only one-third to one-half as much SIII as would be expected if the transforming principle were the only determining influence, as had appeared to be the case from previous studies. The nature of these modifying factors is unknown but it may be suggested that the diminution in SIII production may be caused by deficiency in the formation of a component or components of the polysaccharide, or to the presence of inhibitors of polysaccharide synthesis at some stage of its production.

It has been shown (3) that the virulence of strains of type III pneumococci for mice is correlated with the quantity of SIII formed *in vitro*. A similar relationship was observed in the present experiments, even though the cause of the decreased SIII production differs from that previously reported.

SUMMARY

Pneumococci can possess the normal S transforming principle and yet produce amounts of capsular polysaccharide much less than would be expected if the quantity formed depended only on the nature of the S transforming principle.

BIBLIOGRAPHY

1. MacLeod, C. M., and Krauss, M. R., *J. Exp. Med.*, 1947, **86,** 439.
2. Taylor, H. E., *J. Exp. Med.*, 1949, **89,** 399.
3. MacLeod, C. M., and Krauss, M. R., *J. Exp. Med.*, 1950, **92,** 1.
4. Mørch-Lund, E., *Acta path. et microbiol. scand.*, 1949, **26,** 709.
5. Bernheimer, A. W., personal communication.
6. Heidelberger, M., MacLeod, C. M., Markowitz, H., and DiLapi, M., *J. Exp. Med.*, 1951, **94,** 359.