LOSS OF GROUP CARBOHYDRATE DURING MOUSE PASSAGES OF A GROUP A HEMOLYTIC STREPTOCOCCUS

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(Received for publication, April 6, 1945)

One of the most constant of the known antigenic components of beta hemolytic streptococci is the group-specific carbohydrate—so called C substance. In a given strain, although colony form, hemolysin production, fibrinolysin production, content of type-specific agglutinogens and precipitinogens, and pigment production may be altered under certain circumstances, the persistence of the group carbohydrate is remarkably constant. In so far as is known, only one instance to the contrary has previously been recorded. Reich (1) reported that a change in group carbohydrate could easily be produced in the course of animal passage. This is not in agreement with general experience.

The present report concerns a group A type 27 strain of hemolytic streptococcus which, in the course of passage through mice, lost all detectable traces of its group A carbohydrate without losing its type-specific antigens, without acquiring the carbohydrate of another group, and without changing other recognizable biological characteristics.

The strain was originally sent to this laboratory by Griffith as a representative of type 27, designated by him "780 Tate" and called here "T27." It had been dried from the frozen state according to the technique of Swift (2) and kept for several years. The original strain was very low in mouse virulence and induced in rabbits only a weakly reacting anti-M precipitating serum. Several attempts had been made to increase its virulence by mouse passage but without striking success. The final member of one of these series of mouse passages was frozen and dried as T27/23/2. This culture was subsequently taken out of the dried state and started on another series of mouse passages. After seven direct mouse-to-mouse passages a rather marked increase in virulence occurred. To be sure that a contaminating strain had not been introduced, it was subjected to serological classification and was found by precipitin test to be type 27 but to contain no group-specific carbohydrate for groups A to M. This anomalous strain was called T27/30 (A).

An attempt to repeat the phenomenon was made by returning to the frozen and dried strain, T27/23/2, and starting another series of mouse passages. An increase in virulence was reached after 23 additional mouse-to-mouse passages but no loss of group carbohydrate occurred. This strain was called T27/46/2.

The complete absence of group carbohydrate in the anomalous variant was established by preparing highly concentrated extracts of T27/30 (A) according (*a*) to the Lancefield hydrochloric acid extraction method, and (*b*) to the formamide method of Fuller, and testing serial dilutions of these extracts against potent group A sera. No reaction occurred. When potent group A antisera were absorbed with packed streptococcal cells of this anomalous strain, a small reduction in antibody occurred. This was attributed to a non-specific adsorption. When, on the other hand, packed organisms of T27/46/2 were used for absorption, the group A antibodies were easily and completely removed. If T27/30 (A) was used to immunize rabbits in the manner customary for preparing grouping sera (3), no group antibodies were obtained, although type-specific antibodies were produced. When strain T27/46/2 was similarly employed, very potent group A antisera resulted, and these sera also contained type 27 antibodies.

The cultural characteristics of the virulent anomalous strain (T27/30 (A)) resembled very closely those of the parent strain (T27/23/2) as well as those of the original Griffith strain (T27) and of the virulent carbohydrate-containing strain (T27/46/2). In order, however, to demonstrate with the greatest possible certainty the relationship of the anomalous strain to the earlier strains, studies were made to determine whether the various strains belonged to the same type.

Precipitin Studies.—By the routine precipitin typing technique of Lancefield (3) using M extracts of the organisms and absorbed type-specific sera for the available types, all four strains were found to be type 27.

Antisera prepared by immunizing rabbits with strain T27/46/2, and absorbed with a heterologous strain reacted with M extracts of all four type 27 strains, but not with extracts of strains of other types.

When antisera prepared by immunizing rabbits with T27/46/2 were absorbed respectively by each of the other three strains, all type-specific precipitins were removed from the sera.

Similarly, antisera prepared by immunizing with the anomalous strain T27/30 (A) contained antibodies reacting with M extracts of the other type 27 strains and removable by absorptions with packed cells of any of them.

Thus all four strains were shown to contain the same type-specific precipitinogen on the basis of precipitin typing and reciprocal precipitin absorption tests.

Macroscopic Agglutination Studies.—Three of the type 27 strains were used in these studies. Suspensions of the organisms of strains T27, T27/46/2, and T27/30 (A) were all agglutinated by sera produced by immunizing rabbits with both T27/46/2 and T27/30 (A) after absorption of the sera with a heterologous strain (T14/25/2), but did not agglutinate in sera prepared with this heterologous strain.

TABLE I

Reciprocal Passive Protection Tests in Mice with Strain T27/46/2 and Its Derivative, Strain T27/30 (A)

Sera	Mice inoculated with			
	Dose	Strain T27/46/2	Strain T27/30 (A)	Strain D58/40/1 (type 3)
	cc.			
Type 27 made by inoculating with T27/46/2	10^{-1}	D 1	D 1	D 1
	10^{-2}	S	D 1	D 1
	10^{-3}	S	S	D 1
	10^{-4}	S	S	D 1
	10^{-5}	S	S	D 1
	10^{-6}	S	S	D 1
	10^{-7}	S	S	D 1
	10^{-8}	S	S	D 1
Type 27 (anomalous) made by inoculating with T27/30 (A)	10^{-1}	D 1	D 1	D 1
	10^{-2}	D 1	D 1	D 1
	10^{-3}	D 7	D 9	D 1
	10^{-4}	S	S	D 1
	10^{-5}	S	S	D 1
	10^{-6}	S	S	D 1
	10^{-7}	S	S	D 1
	10^{-8}	S	S	D 1
Type 3 made by inoculating with D58/40/1	10^{-1}	D 1	D 1	D 1
	10^{-2}	D 1	D 1	S
	10^{-3}	D 1	D 3	S
	10^{-4}	D 1	D 7	S
	10^{-5}	D 2	D 3	S
	10^{-6}	D 2	S	S
	10^{-7}	D 3	S	S
	10^{-8}	S	S	S
Normal rabbit serum	10^{-1}	D 1	D 1	D 1
	10^{-2}	D 2	D 2	D 1
	10^{-3}	D 1	D 1	D 1
	10^{-4}	D 2	D 1	D 1
	10^{-5}	D 1	D 2	D 1
	10^{-6}	D 2	D 3	D 1
	10^{-7}	D 2	D 2	D 1
	10^{-8}	S	S	D 1
Control No serum	10^{-1}	D 1	D 1	D 1
	10^{-2}	D 1	D 1	D 1
	10^{-3}	D 1	D 1	D 1
	10^{-4}	S	D 2	D 1
	10^{-5}	D 3	D 2	D 1
	10^{-6}	D 2	D 2	D 1
	10^{-7}	D 2	S	D 1
	10^{-8}			

D with numeral indicates death within that number of days.

S indicates survival for at least 2 weeks.

Furthermore, sera prepared against T27/46/2 and absorbed with packed cells of T27/46/2, T27, or T27/30 (A) had all of their type 27 agglutinins removed. Similarly, sera prepared against T27/30 (A) lost their type 27 agglutinins on absorption with packed cells of T27, T27/46/2, or T27/30 (A) but not when absorbed with packed cells of T14/25/2.

Thus it was further shown by macroscopic agglutinations and reciprocal agglutinin absorptions that all three strains contained the same type-specific agglutinogens.

Protection Tests.—The two mouse-virulent strains T27/46/2 and T27/30 (A) and their corresponding antisera were used for protection tests. It was found that antisera from either of the strains would protect mice against both of them but not against a virulent type 3 strain; this indicates that the two strains contained similar antigens capable of inducing protective antibodies in rabbits. (Table I.)

On the basis, therefore, of precipitin typing, reciprocal precipitin absorption tests, agglutinations, reciprocal agglutinin absorption tests, and protection tests, it is established that the strains T27/46/2 and T27/30 (A) were derived from T27/23/2 which in turn was derived from the original T27 strain.

SUMMARY

In the course of rapid passages through mice a strain of group A type 27 hemolytic streptococcus was found to have lost its group carbohydrate without the loss of type-specific precipitinogens, agglutinogens, or its capacity to induce protective antibodies in rabbits, and without the acquisition of the carbohydrate of another group.[1]

The loss of group carbohydrate was shown to be complete, within the limits of the methods for its detection. Extracts of the anomalous strain did not react with group A antisera; and antisera prepared with organisms of this anomalous strain did not contain demonstrable antibodies for the group carbohydrate. Bacterial suspension of the anomalous strain failed to absorb any appreciable amount of group-specific antibody.

The fact that the anomalous strain lacking group-specific carbohydrate, C, was derived from the original was established by the demonstration of persistence of its other characteristics, in particular the precipitinogens, agglutinogens, and antigens responsible for protective antibodies of type 27.

BIBLIOGRAPHY

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[1] Similar variants of 2 other strains of group A hemolytic streptococci, which have lost their group-specific carbohydrate, C, during serial mouse passage, have since been encountered in this laboratory. Neither strain belonged to a known type, and not enough further work has been done to prove the identity of the variants with the corresponding original strains.