SEROLOGICAL RELATIONSHIP BETWEEN THE MENINGOCOCCI OF THE FRENCH TYPE C AND GROUP II ALPHA

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As a result of early serological studies of *Neisseria meningitidis* that were made during World War I, it was established readily that Types A and B of the French classification (Nicolle *et al.*, 1918) corresponded with Types I and II of British reports (Gordon and Murray, 1915; Griffith, 1916; Scott, 1916). The French Types C and D were rare and were not found to be related to any reported British types.

During more than 3 decades since those designations were introduced, nomenclature of the meningococci has undergone changes: Types I and III were recognized as belonging to the single broad Group I; strains designated as II *alpha* (Cohen, 1940; Branham, 1942) emerged as a definite entity from the less specific broad Group II; while "Type IV", as well as the French C and D, seemed to disappear altogether.

In 1948 a Subcommittee of the International Committee on Bacteriological Nomenclature proposed a revision of the classification of meningococci in accordance with the recommendation of the International Bacteriological Code of Nomenclature (Buchanan *et al.*, 1948) which suggests that serological groups be designated by capital letters and types within the groups by arabic numerals. In this revision the Subcommittee suggested that Groups I and II be designated as A and B, Group II *alpha* as C, and IV as D (Preliminary Report Subcommittee, 1950; Branham, 1953). An objection to this proposed classification was that these new Groups C and D might be confused with the older French Types C and D, the relationships of which were completely unknown. Since the availability of strains of these old types was unknown to members of the committee at the time of their report, clarification of this situation seemed unlikely.

MATERIALS AND METHODS

During August, 1951, however, the authors of this report received three strains of meningococcus, said to represent the old French Type C, from Dr. J. L. Chevé of the Pasteur Institute Annexe in Dordogne, France. These were labeled C 107, C 109, and C 113 and were accompanied by a small amount of "C" antiserum. A few months later three other strains, C 1, C 2, C 3, were obtained through Dr. Hauduroy of the Lausanne catalog from Dr. Thibault of the Pasteur Institute in Paris.

These six strains were subjected first to the usual laboratory routine; i.e., were plated on rabbit blood agar, three to five colonies picked to blood agar slants, and the resulting cultures together with the original culture studied in respect to morphology, colonial appearance, fermentation reactions, and agglutination with polyvalent antimeningococcus serum.

RESULTS

Cultures from all six strains showed typical neisserian morphology and were well agglutinated by polyvalent antimeningococcus serum. Colony appearance was more nearly that of old stock cultures than that of freshly isolated ones. This "stock" appearance was especially true of the three Paris strains which seemed quite "rough"; two of these failed to show typical fermentation.

Typing was undertaken with monovalent group sera: I, II, and II *alpha*. Three of the six C strains were agglutinated only by Group II *alpha* serum; the other three, although agglutinated by II *alpha* serum, were agglutinated also by Group II serum, and one of these, C 3, by Group I serum also. Since there are common somatic antigens in all meningococci, cross agglutination is found commonly with strains that have lost their specific capsular substance.

An attempt was made to restore some degree of smoothness to these strains by mouse passage. At first, tremendous numbers of meningococci were required to induce a generalized infection: 5 hour growth from blood agar slants was suspended in 5 per cent gastric mucin and injected into mice intraperitoneally, each mouse receiving

1 ml containing meningococci from approximately 1½ slants. Cultures were made from the heart blood of the mice that were sacrificed within 24 hours after injection. As consecutive passages were made, the number of meningococci required to induce an infection became less, and it was possible to estimate the number of bacteria given. Using a suspension diluted to contain 2 billion bacteria per ml as a starting point, these strains were brought to a state in which 1 ml of a 10^{-3} suspension in 5 per cent mucin (2,000,000 meningococci) killed 16 to 20 g mice within 40 hours. The Dordogne strains were given only two passages, but the remaining three were given six.

All six C strains were now restudied in respect to colonial form and fermentation. Colonies of five strains were definitely larger, more uniform in size, and more moist. Acid production from glucose and maltose also occurred in normal fashion. Strain C 3 proved refractory in all of these respects; there was little difference in colonial appearance, and no fermentation of glucose and maltose occurred. Since massive doses of this strain had failed to kill mice until the third passage, and 200,000,000 meningococci were required to kill in the sixth passage, it may be concluded that this strain C 3 had become more degraded than the others. Since some improvement was obtained with C 3, it is possible that further repeated passage might have shown additional change.

Preliminary agglutination tests had indicated a relation between these C meningococci and those of Group II *alpha*. Such studies were now continued with the rejuvenated C cultures. Results are shown in table 1 in which the six C

TABLE 1

Agglutination of C strains by monovalent group sera

STRAIN	SERA AND DILUTIONS																SALINE CONTROL
	Group I				Group II				Group IIα				Polyvalent				
	20	40	80	160	20	40	80	160	20	40	80	160	40	80	160	320	
1027 I	4	3	2	0	0	0	0	0	0	0	0	0	4	4	4	4	1
2121 II	0	0	0	0	3	2	1	0	0	0	0	0	4	4	4	3	0
2104 IIα	0	0	0	0	1	0	0	0	4	4	4	1	4	4	4	4	0
C 107	0	0	0	0	0	0	0	0	3	2	2	1	1	2	3	4	0
C 113	0	0	0	0	1	0	0	0	4	4	3	1	3	4	4	4	0
C 2	0	0	0	0	0	0	0	0	4	3	3	2	0	2	3	3	0
C 109	0	0	0	0	2	2	0	0	3	3	2	1	1	1	1	2	0
C 1	0	0	0	0	3	2	1	0	3	3	2	1	4	4	3	2	0
C 3	2	2	1	1	3	3	2	1	3	2	1	1	0	2	2	2	0

TABLE 2

*Agglutination of French C strains by many II alpha sera**

ANTIGEN	SERA AND DILUTIONS																								SALINE CONTROL
	1054 IIα				1171 IIα				1883 IIα				1902 IIα				1908 IIα				2104 IIα				Polyvalent			
	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	
C 107	3	3	2	1	3	3	2	2	4	3	2	1	3	2	2	1	4	3	1	1	4	4	3	2	4	3	2	2	0
C 109	3	2	0	1	4	3	2	1	1	1	1	1	4	2	1	1	3	2	1	1	3	3	2	2	3	2	2	2	1
C 113	4	4	1	0	4	3	1	0	4	4	3	1	4	4	1	1	3	2	2	1	4	4	3	2	3	2	2	2	0
C 1	3	2	2	1	3	3	2	2	2	2	1	1	4	3	1	1	3	4	2	1	3	3	1	1	1	2	4	3	0
C 2	4	3	2	1	4	4	3	1	4	3	1	1	4	3	1	1	4	3	1	1	1	1	1	1	3	4	4	3	0
C 3	1	1	1	0	4	4	3	1	2	1	1	1	3	1	1	1	4	3	2	1	3	3	2	1	2	2	3	2	1
1027 I	1	0	0	0	4*	3	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	—	—	—	1
2092 II	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	—	—	—	—	1
2104 IIα	4	2	1	1	4	4	3	1	4	2	2	1	4	3	2	2	4	3	2	2	4	4	2	1	4	4	2	1	1

* This crossing of IIα strain 1171 with Group I is a characteristic that is constant for this one strain.

TABLE 3

Agglutination of French C and II alpha meningococci by sera of rabbits immunized with French C strains

ANTIGEN	SERA AND DILUTIONS																								SALINE CONTROL
	C 107				C 109				C 113				C 1				C 2				C 3				Polyvalent				
	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	20	40	80	160	
C 107	3	3	3	2	2	2	3	1	3	2	2	2	2	2	2	2	3	3	3	2	3	3	3	2	4	2	2	2	1
C 109	3	2	2	1	4	4	3	3	3	3	2	2	3	2	2	1	3	1	1	0	3	3	2	1	4	3	3	2	0
C 113	3	3	2	1	2	2	2	2	3	3	2	2	3	3	2	2	3	3	3	2	4	3	3	3	4	4	3	3	1
C 1	3	3	3	1	2	2	2	1	3	2	1	1	3	3	3	3	4	4	3	2	4	4	4	3	2	2	2	1	1
C 2	3	3	3	2	3	3	3	2	3	3	3	2	2	2	3	2	3	3	4	3	4	4	4	4	1	1	1	1	1
C 3	4	3	2	2	3	2	2	1	3	2	2	1	2	2	2	2	3	3	2	2	4	4	3	4	3	2	2	2	1
1852 IIα	3	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	4	3	2	2	3	2	1	1	4	4	3	2	1
1860 IIα	3	1	1	1	3	1	1	1	4	4	3	0	3	0	0	0	1	1	1	1	1	0	0	0	3	3	2	2	0
1861 IIα	4	4	1	1	4	3	1	1	4	4	4	2	3	3	1	1	3	2	1	1	1	1	0	0	3	3	3	2	1
1874 IIα	4	2	1	1	4	1	1	1	4	4	1	1	1	1	1	1	4	3	2	1	3	2	1	1	3	3	3	2	1
1880 IIα	3	1	0	0	2	1	1	1	4	3	2	1	4	3	1	1	3	2	2	0	2	1	0	0	4	3	2	2	0
1882 IIα	4	4	3	2	4	3	2	2	4	4	4	3	2	1	1	1	4	3	2	1	1	1	1	1	3	3	3	2	1
1896 IIα	3	2	2	2	2	2	1	1	2	1	1	1	3	4	3	3	4	4	3	2	3	3	2	2	4	3	2	2	1
1902 IIα	3	2	2	1	3	1	1	1	4	4	3	1	3	2	1	1	4	3	3	3	1	1	1	1	3	4	3	2	1
1973 IIα	4	3	2	1	4	3	2	1	4	3	2	1	3	3	2	2	4	4	4	2	4	4	3	2	2	2	2	3	1
2104 IIα	4	4	2	1	4	3	1	0	4	4	3	1	2	1	1	1	1	1	0	0	1	1	0	0	4	2	2	1	0
2092 II	0	0	0	0	0	0	0	0	2	2	0	0	2	0	0	0	2	0	0	0	2	0	0	0	4	3	3	2	0
1027 I	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	0

TABLE 4

Agglutination of II alpha and French C strains by French C serum (Dordogne)

ANTIGEN STRAIN	SERA AND DILUTIONS																				SALINE CONTROL
	Polyvalent				NIH I				NIH II				NIH IIα				French C (Dordogne)				
	40	80	160	320	20	40	80	160	20	40	80	160	20	40	80	160	80	160	320	640	
C 107	2	2	3	4	0	0	0	0	0	0	0	0	3	2	2	0	4	4	3	2	0
C 109	2	2	2	2	0	0	0	0	0	0	0	0	3	2	0	0	4	3	3	3	0
C 113	3	4	4	4	0	0	0	0	0	0	0	0	4	4	3	0	4	4	3	2	0
C 1	0	0	2	2	0	0	0	0	1	1	0	0	3	2	2	0	4	4	3	2	0
C 2	0	2	3	3	0	0	0	0	0	0	0	0	4	3	3	2	4	4	3	3	0
C 3	0	0	2	2	0	0	0	0	3	3	2	0	2	2	0	0	4	4	3	3	0
2109 IIα	3	2	2	2	0	0	0	0	0	0	0	0	2	3	2	0	4	4	3	2	0
2140 IIα	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	4	3	2	0	0
1375 IIα	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	3	3	0	0	0
2104 IIα	4	4	4	3	0	0	0	0	0	0	0	0	4	4	0	0	4	4	4	2	0
1628 IIα	2	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	4	4	2	0	0
2133 IIα	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	4	3	1	0	0
2144 IIα	2	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	4	3	0	0	0
2123 I	3	2	0	0	4	4	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0
2127 II	3	3	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0

strains together with representative cultures of Groups I, II, and II *alpha* were tested for agglutination with monovalent Group I, Group II, and Group II *alpha* sera as well as with a polyvalent serum. All six strains were agglutinated by II *alpha* serum. With strains C 107, C 113, and C 2 cross agglutination was negligible. Some crossing with Group II occurred with C 109, C 1, and C 3 although C 109 and C 1 showed a higher titer with Group II *alpha*. C 3 was agglutinated by all sera.

Similar tests were set up using a number of

sera prepared from individual II *alpha* strains in order to ascertain if relation to Group II *alpha* were a regular occurrence and not merely a property of one serum. A polyvalent serum was always included as a control on the agglutinability of the antigen suspensions, as well as representative strains of Groups I, II, and II *alpha* as a check upon the specificity of the sera. All of the French C antigens were agglutinated by most of the II *alpha* sera. There is great variation in the antigenic pattern of II *alpha* strains, so that it was not surprising that all II *alpha* sera did not agglutinate all French C cultures equally well. Strains C 109 and C 3 were agglutinated by fewer sera than were the other four strains. This agglutination by several II *alpha* sera is shown in table 2.

Rabbits were immunized with each of the six C strains by a series of intravenous injections. Table 3 shows agglutination, by these sera, of the six homologous C strains and of a number of II *alpha* strains, as well as representatives of Groups I and II which were included to check specificity. Each of these six sera agglutinated all of the C strains and most of the II *alpha* strains that were included in the test. Although C 3 was still a "rough" culture and was atypical in its fermentation characteristics, it obviously was related very closely serologically to the other five strains representing that group, but it failed to agglutinate 50 per cent of the II *alpha* strains included.

Reference has been made above to a sample of serum representing the French Type C that was received from Dr. Chevé of Dordogne. A number of strains of meningococci of various serological groups was tested for agglutinability with this serum, as well as with a polyvalent serum and sera representing Groups I, II, and II *alpha*. Results, as shown in table 4, leave no doubt as to the close relation between the French Type C and the Group II *alpha* and indicate that these strains should be placed together in one group.[1]

[1] After this report had gone to press, two additional strains of *N. meningitidis*, Type C, were received from Dr. Thibault of the Pasteur Institute in Paris. These were found, likewise, to be indistinguishable from those designated as of Group II *alpha*.

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SUMMARY

Six strains of the "Type C" meningococcus of the French classification have been studied in relation to the serological groups found in the United States during recent years. Five of these six C strains have been found to correspond with those designated as Group II *alpha*. The sixth strain had become too "rough" to be studied satisfactorily; its colonies had become atypical, it had lost its ability to ferment any sugars, and it cross agglutinated with sera of all groups. That a relationship of this sixth strain to Group II *alpha* had at some time existed is suggested by the fact that serum prepared by immunizing rabbits with it agglutinated the other five C strains very well, though agglutination of II *alpha* strains was less regular.

In this study the close relation between the French Type C and Group II *alpha* is shown clearly. The recommendation of the Subcommittee on the Nomenclature of the *Neisseriaceae* that Group II *alpha* be designated as Group C is justified by the evidence presented.

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