IMMUNITY TO *HAEMOPHILUS INFLUENZAE* TYPE B: THE ROLE OF THE CAPSULAR ANTIBODY

Y. MPAIRWE*

*Department of Medical Microbiology, Makerere University,
Kampala, Uganda*

It is believed that the protective antibody against *Haemophilus influenzae* type b is that produced against its capsular antigen (Alexander, 1965; MacLeod and Bernheimer, 1965; Davis *et al.*, 1967). If this is so we should expect the age-distribution of this capsular antibody to resemble that of the bactericidal factor in the blood against the causative organism (Fothergill and Wright, 1933) and to be inversely related to that of *H. influenzae* type-b infections.

In the present study the frequency of the capsular antibody and the bactericidal factor at various ages was determined. The purpose of this paper is to report that they were found to be dissimilar, and to suggest that the belief that immunity to *H. influenzae* type-b infections is due to capsular antibody may be incorrect.

MATERIALS AND METHODS

Serum samples were obtained from the blood collected aseptically from patients in Mulago Hospital, Kampala, who were not on antibiotic treatment. Their ages ranged from birth (cord blood) to adulthood. Sera were stored at -20°C and used within 4 wk of collection.

The *H. influenzae* type b strain used was isolated from cerebrospinal fluid. It formed iridescent colonies on Levinthal's agar and was agglutinated by type-b antiserum (Burroughs Wellcome & Co.) and was not agglutinated by antisera against any other types. *Diluent* for emulsifying *H. influenzae* type b in bactericidal tests was 10 per cent. normal rabbit or bovine serum (previously heated at 56°C for 30 min.) in saline. "*Suspension - 4*" used in studies of the bactericidal factor, was made by emulsifying, in 5 ml of diluent, two colonies of *H. influenzae* type b from an 18-hr culture on chocolate agar and transferring 0.1 ml of this emulsion into 0.9 ml of diluent. From this, further dilutions in diluent were made to 10^{-4} . *Drops* (0.02 ml) were those delivered by a calibrated Pasteur pipette.

Capsular antibody was detected by the haemagglutination technique of Turk and Green (1964). Sera were tested at two-fold dilutions in saline from undiluted to 1 in 64 inclusive. Sera in which antibody was detected at any titre were recorded as positive. The *bactericidal factor* was detected as follows. One drop of "*suspension - 4*" was added to 0.2 ml of the test serum in a screw-capped 5-ml bottle. This was shaken gently and incubated at 37°C in a waterbath for 45 min. At the end of this period it was shaken again and a drop plated on to chocolate agar and incubated for 18-24 hr at 37°C . Plates were then examined for growth, and serum samples from which there was no growth were recorded as positive for bactericidal factor.

RESULTS

The capsular antibody was looked for in 132 subjects and its age-distribution in them is shown in the table. It was uncommon in all age-groups below 5 yr

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* Present address: Department of Clinical Pathology, University College Hospital, London, W.C.1.

including newborn babies, and was detected in only 30 per cent. of subjects over the age of 5 yr. The bactericidal factor was looked for in a smaller number of subjects. It was possessed by a large proportion of newborn babies and of subjects over 5 yr of age, but it was absent or uncommon in age-groups between 2 mth and 5 yr (table).

DISCUSSION

The age-distribution of *Haemophilus influenzae* meningitis was shown by Fothergill and Wright (1933) to be inversely related to that of a bactericidal factor against the causative organism found in the blood. This finding was used to support their hypothesis that immunity in the newborn is acquired from the mother transplacentally, lost within the first few weeks of birth and gradually built up through inapparent infection, immunity generally being poorly

TABLE
*Age-distribution of capsular and bactericidal antibodies
against Haemophilus influenzae type b*

Age of serum donor	Number of sera			
	examined for capsular antibody	containing capsular antibody (with percentage)	examined for bactericidal antibody	containing bactericidal antibody (with percentage)
At birth	31	1 (3)	8	7 (88)
2 mth-2 yr	43	0 (0)	11	0 (0)
2-5 yr	28	2 (8)	10	4 (40)
Over 5 yr	30	9 (30)	13	11 (85)

developed before the age of 3-4 yr. This explanation for the now well-known age incidence of *H. influenzae* type b infections has been generally accepted (e.g., Weinstein, 1946; Smith, 1956; Mathies, Hodgman and Ivler, 1965; Jawetz, Melnick and Adelberg, 1966; Collier, Connor and Nyhan, 1967).

It has also been stated, however, that the protective antibody against *H. influenzae* type b is that produced against its capsular antigen (Alexander, 1965; MacLeod and Bernheimer, 1965; Davis *et al.*, 1967). But if this were so, the age-distribution of the capsular antibody would be expected to be, like that of the bactericidal factor, inversely related to that of *H. influenzae* type b infections. To the best of my knowledge, this has not been reported to be the case and the present studies do not indicate that it is so. The only *H. influenzae* antibodies that have been shown to have a similar age-distribution to that of the bactericidal factor are those produced against a somatic antigen (Tunevall, 1953). That a somatic antigen could provoke protective antibody formation, in the rabbit at least, was demonstrated by Dubos (1942).

Observations in orphan homes where the carriage rate for *H. influenzae* type b is sometimes very high (Turk, 1963; Mpairwe, 1970) have suggested

that in ordinary open communities, *H. influenzae* type b is rarely communicated from one household to another (Mpairwe). Hence only a very small proportion of children would be expected to have come into contact with this serotype by the age of 5 yr, and this might explain the low frequency of the capsular antibody in the present study. Yet from the age-incidence of *H. influenzae* type-b infections (Turk and May, 1967) it appears that by the age of 5 most children have already developed immunity. There would seem, therefore, to be a discrepancy between the rate at which immunity develops and that at which *H. influenzae* type b appears to circulate in these communities, suggesting that whatever it is that immunises against *H. influenzae* type b, it circulates faster than this serotype.

In view of this, the belief that the protective antibody against *H. influenzae* type b is that produced against its capsular antigen—a belief that is largely based on analogies (Alexander; Davis *et al.*) between capsulated *H. influenzae* and capsulated pneumococci—may be erroneous.

SUMMARY

Sera were tested for capsular antibody and for bactericidal factor against *Haemophilus influenzae* type b. The age-distribution of the bactericidal factor corresponded to the known age-distribution of immunity to serious infection with *H. influenzae* type b, but that of the capsular antibody did not. This finding argues against the belief that the protective antibody to *H. influenzae* type-b infections is that produced against its capsular antigen.

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